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I, Susan ANTHONY BA, ACIS,

Director of RWS Group Ltd, of Europa House, Marsham Way, Gerrards Cross, Buckinghamshire, England declare;

1. That I am a citizen of the United Kingdom of Great Britain and Northern Ireland.

2. That the translator responsible for the attached translation is well acquainted with the German and English languages.

3. That the attached is, to the best of RWS Group Ltd knowledge and belief, a true translation into the English language of the accompanying copy of the specification filed with the application for a patent in Germany on 29 April 2002 under the number 102 19 203.0 and the official certificate attached hereto.

4. That I believe that all statements made herein of my own knowledge are true and that all statements made on information and belief are true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent application in the United States of America or any patent issuing thereon.

For and on behalf of RWS Group Ltd

The 31st day of May 2006

FEDERAL REPUBLIC OF GERMANY

[Eagle crest]

Priority Certificate

for the filing of a Patent Application

File Reference:	102 19 203.0
Filing date:	29 April 2002
Applicant/Proprietor:	BASF Plant Science GmbH, Ludwigshafen/DE
Title:	Method for the production of polyunsaturated fatty acids in plants
IPC:	A 01 H 1/00

The attached documents are a correct and accurate reproduction of the original submission for this Application.

Munich, 25 November 2002

German Patent and Trademark Office

The President

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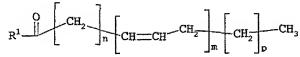
Weihmayr

We claim:

1. A process for the production of compounds of the general formula I:



(I)

in transgenic plants with a content of at least 1% by weight based on the total fatty acids, which process comprises the following steps:

a) introducing, into a plant, at least one nucleic acid sequence which encodes a polypeptide with an Δ6-desaturase activity; and

b) introducing at least one second nucleic acid sequence which encodes a polypeptide with a Δ6-elongase activity; and,

c) if appropriate, introducing a third nucleic acid sequence which encodes a polypeptide with a Δ5-desaturase activity;

d) followed by growing and harvesting the plants; and

where the variables and substituents in the formula I have the following meanings:

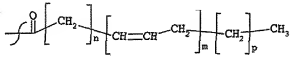
R^1 = -OH, coenzyme A (thioester), phosphatidylcholine, phosphatidylethanolamine, phoshatidylglycerol, diphosphatidylglycerol, phosphatidylserine, phosphatidylinositol, sphingolipid, glycoshingolipid or a radical of the following general formula II

(II)

R^2 = H, phosphatidylcholine-, phosphatidylethanolamine-, phosphatidylglycerol-, diphosphatidylglycerol-, phosphatidylserine-, phosphatidylinositol-, shingolipid-, glycoshingolipid-, glycosphingolipid- or saturated or unsaturated C_2–C_{24}–alkylcarbonyl-,

R^3 = H, saturated or unsaturated C_2–C_{24}–alkylcarbonyl-, or

R^2 and R^3 independently of one another represent a radical of the general formula Ia



(Ia),

n = 3, 4 or 6, m = 3, 4 or 5 and p = 0 or 3.

2. The process according to claim 1, wherein the substituents R^2 and R^3 independently of one another are C_{10}–C_{22}–alkylcarbonyl-.

3. The process according to claim 1 or 2, wherein the substituents R^2 and R^3 independently of one another are C_{16}-, C_{18}-, C_{20}- or C_{22}-alkylcarbonyl-.

4. The method according to any of claims 1 to 3, wherein the substituents R^2 and R^3 independently of one another are unsaturated C_{16}-, C_{18}-, C_{20}- or C_{22}-alkylcarbonyl- with one, two, three, four or five double bonds.

5. The method according to any of claims 1 to 4, wherein the transgenic plant is an oil crop.

6. The method according to any of claims 1 to 5, wherein the transgenic plant is selected from the group consisting of soya, peanut, oilseed rape, canola, linseed, evening primrose, verbascum, thistle, hazelnut, almond, macadamia, avocado, bay, wild roses, pumpkin/squash, pistachios, sesame, sunflower, safflower, borage, maize, poppy, mustard, hemp, castor-oil plant, olive, Calendula, Punica, oil palm, walnut or coconut.

7. The method according to any of claims 1 to 6, wherein the compounds of the formula I are obtained from the transgenic

plants in the form of their oils, fats, lipids or free fatty acids by pressing or extraction.

8. The process according to any of claims 1 to 7, wherein the oils, fats, lipids or free fatty acids obtained as claimed in claim 7 are refined.

9. The process according to any of claims 1 to 8, wherein the saturated or unsaturated fatty acids present in the compounds of the formula I are liberated.

10. The method according to any of claims 1 to 9, wherein the saturated or unsaturated fatty acids are liberated by alkaline hydrolysis or enzymatic cleavage.

11. The method according to any of claims 1 to 10, wherein the compounds of the general formula I are present in the transgenic plant at a content of at least 5% by weight, based on the total fatty acids.

12. The process according to any of claims 1 to 11, wherein the nucleic acid sequences which encode the polypeptides with Δ6-desaturase activity, Δ6-elongase activity or Δ5-desaturase activity are selected from the group consisting of:

a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29 or SEQ ID NO: 31,

b) nucleic acid sequences which, owing to the degeneracy of the genetic code, are obtained by back translation of the amino acid sequences shown in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30 or SEQ ID NO: 32,

c) derivatives of the nucleic acid sequences shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29 or SEQ ID NO: 31 which encode polypeptides with the amino acid sequences shown in SEQ ID NO: 2, SEQ ID NO: 4,

SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30 or SEQ ID NO: 32 and which have at least 50% homology at the amino acid level, without the enzymatic activity of the polypeptide being substantially reduced.

13. The process according to any of claims 1 to 12, wherein the nucleic acid sequences as claimed in claim 8 are linked with one or more regulatory signals in a nucleic acid construct.

14. The method according to any of claims 1 to 13, wherein the nucleic acid construct comprises additional biosynthetic genes of the fatty acid or lipid metabolism selected from the group consisting of acyl-CoA dehydrogenase(s), acyl-ACP [= acyl carrier protein] desaturase(s), acyl-ACP thioesterase(s), fatty acid acyl transferase(s), fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenases, lipoxygenases, triacylglycerol lipases, allene oxide synthases, hydroperoxide lyases or fatty acid elongase(s).

Method for the production of polyunsaturated fatty acids in plants

Description

The present invention relates to a method for the production of fatty acid esters which comprise unsaturated fatty acids with at least three double bonds, and to free unsaturated fatty acids with a content of at least 1% by weight based on the total fatty acids present in the plants, by expressing at least one nucleic acid sequence which encodes a polypeptide with Δ6-desaturase activity and at least one nucleic acid sequence which encodes a polypeptide with Δ6-elongase activity. Advantageously, these nucleic acid sequences can, if appropriate, be expressed in the transgenic plant together with a third nucleic acid sequence which encodes a polypeptide with Δ5-desaturase activity.

The invention furthermore relates to the use of defined nucleic acid sequences which encode polypeptides with a Δ6-desaturase activity, Δ6-elongase activity or Δ5-desaturase activity selected from a group of nucleic acid sequences, and/or to the use of nucleic acid constructs comprising the abovementioned nucleic acid sequences.

Certain products and by-products of naturally occurring metabolic processes in microbial cells or in the cells of animals and, advantageously plants, have utility for a wide range of industries, including the feed, food, cosmetics and pharmaceutical industries. These molecules, which are collectively termed "fine chemicals", also include, for example, lipids and fatty acids, one representative class of which are the polyunsaturated fatty acids. Polyunsaturated fatty acids (PUFAs) are added for example to infant formula for increasing the nutritional value of these foods. PUFAs have, for example, a positive effect on the cholesterol level in the blood of humans and are therefore useful for protection against heart disease. Fine chemicals such as polyunsaturated fatty acids (PUFAs) can be isolated from animal sources such as, for example, fish, or produced by microorganisms by culturing microorganisms which have been developed such that they produce and accumulate or secrete large amounts of one or more desired molecules.

Fatty acids and triglycerides have a multiplicity of uses in the food industry, in animal nutrition, in cosmetics and in the pharmacological sector. Depending on whether they take the form of free saturated or unsaturated fatty acids or triglycerides

with an increased content of saturated or unsaturated fatty acids, they are suitable for a variety of uses. Polyunsaturated Ω 3-fatty acids and Ω 6-fatty acids constitute an important part of animal and human nutrition. Owing to the present-day composition of human nutrition, an addition of polyunsaturated Ω 3-fatty acids, which are predominantly found in fish oils, to the food is of particular importance. Thus, for example, polyunsaturated fatty acids such as docosahexaenoic acid (=DHA, $C22:6^{\Delta 4,7,10,13,16,19}$) or eisosapentaenoic acid (= EPA, $C20:5^{\Delta 5,8,11,14,17}$) is added to baby formula for increasing the nutritional value. DHA is said to have a positive effect on brain development.

The various acids and triglycerides are obtained mainly from microorganisms such as Mortierella or from oil-producing plants such as soybeans, oilseed rape, sunflower, algae such as Cryptocodinium or Phaeodactylum and others, the products being obtained, as a rule, in the form of their triacylglycerides (= triglycerides = triglycerols). However, they can also be obtained from animals such as, for example, fish. The free fatty acids are advantageously prepared by hydrolysis. Higher polyunsaturated fatty acids such as DHA, EPA, arachidonic acid (= ARA, $C20:4^{\Delta 5,8,11,14}$), dihomo-γ-linolenic acid ($C20:3^{\Delta 8,11,14}$) or docosapentaenoic acid (DPA, $C22:5^{\Delta 7,10,13,16,19}$) cannot be isolated from oil crops such as oilseed rape, soybeans, sunflower, safflower or others. Conventional natural sources of these fatty acids are fish such as herring, salmon, sardine, redfish, eel, carp, trout, halibut, mackerel, zander or tuna, or algae.

Depending on the intended purpose, oils with saturated or with unsaturated fatty acids are preferred; thus, for example, lipids with unsaturated fatty acids, specifically polyunsaturated fatty acids, are preferred in human nutrition. The polyunsaturated Ω 3-fatty acids are said to have a positive effect on the cholesterol level in the blood and thus on the possibility of preventing heart disease. The risk of heart disease, stroke or hypertension can be reduced markedly by adding these ω3-fatty acids to the food. Also, Ω 3-fatty acids can have a positive effect on inflammatory processes, specifically chronically inflammatory processes in connection with immunological diseases such as rheumatoid arthritis. These fatty acids are therefore added to foodstuffs, specifically dietetic foodstuffs, or are used in medicaments.

In connection with these rheumatic diseases due to the usual composition of our foods, Ω 6-fatty acids such as arachidonic acid tend to have a negative effect on these diseases.

Ω3- and Ω6-fatty acids are precursors of tissue hormones, what are known as eicosanoids such as the postaglandins, which are derived from dihomo-γ-linolenic acid, arachidonic acid and eicosapentaenoic acid, the thromoxanes and the leukotrienes, which are derived from arachidonic acid and eicosapentaenoic acid. Eicosanoids (known as the PG_2 series), which are formed from Ω6-fatty acids, promote, as a rule, inflammatory reactions, while eicosanoids (known as the PG_3 series) from Ω3-fatty acids have a minor, or no, proinflammatory action.

Owing to the positive properties, there has been no lack of attempts in the past to make available genes which are involved in the synthesis of fatty acids or triglycerides, for the production, in various organisms, of oils with a modified content of unsaturated fatty acids. Thus, WO 91/13972 and its US equivalent describe a Δ9-desaturase. A Δ15-desaturase is claimed in WO 93/11245 and a Δ12-desaturase is claimed in WO 94/11516. Further desaturases are described, for example, in EP-A-0 550 162, WO 94/18337, WO 97/30582, WO 97/21340, WO 95/18222, EP-A-0 794 250, Stukey et al., J. Biol. Chem., 265, 1990: 20144-20149, Wada et al., Nature 347, 1990: 200-203 or Huang et al., Lipids 34, 1999: 649-659. However, the biochemical characterization of the various desaturases is incomplete as yet since the enzymes, being membrane-bound proteins, can only be isolated and characterized with great difficulty (McKeon et al., Methods in Enzymol. 71, 1981: 12141-12147, Wang et al., Plant Physiol. Biochem., 26, 1988: 777-792). As a rule, membrane-bound desaturases are characterized by introduction into a suitable organism which is subsequently analyzed for enzyme activity by means of analyses of the starting material and the product. Δ6-Desaturases are described in WO 93/06712, US 5,614,393, US5614393, WO 96/21022, WO00/21557 and WO 99/27111, and their application for the production in transgenic organisms has also been described, such as in WO98/46763 WO98/46764, WO9846765. In this context, the expression of various desaturases is also described and claimed, as is the case in WO99/64616 or WO98/46776, as is the formation of polyunsaturated fatty acids. As regards the efficacy of the expression of desaturases and their effect on the formation of polyunsaturated fatty acids, it must be noted that only minor contents of Δ6-unsaturated fatty acids/lipids, such as, for example, gamma-linolenic acid and stearidonic acid, have been obtained by expression of a single desaturase, as described to date. Moreover, a mixture of ω 3- and ω 6-fatty acids has been obtained as a rule, since all of the Δ6-desaturases described to date converted for example not only linoleic acid (ω6-fatty

acid), but also α-linolenic acid (ω3-fatty acid).

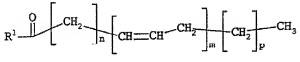
Particularly suitable microorganisms for the production of PUFAs are microorganisms such as Thraustochytrium species or Schizochytrium species, algae such as Phaeodactylum tricornutum or Crypthecodinium species, ciliates such as Stylonychia or Colpidium, fungi such as Mortierella, Entomophthora or Mucor. Strain selection has made possible the development of mutant strains of the microorganisms in question which produce a series of desirable compounds, including PUFAs. The mutation and selection of strains with an improved production of a particular molecule, such as the polyunsaturated fatty acids, is, however, a time-consuming and difficult procedure. This is why recombinant methods are preferred whenever possible, as described above. However, only limited amounts of the desired polyunsaturated fatty acids such as DPA, EPA or ARA can be produced with the aid of the abovementioned microorganisms, these unsaturated fatty acids being obtained, as a rule, as fatty acid mixtures of, for example, EPA, DPA and DHA, depending on the microorganism used.

As an alternative, the production of fine chemicals can suitably be carried out on a large scale via the production in plants which have been developed such that they produce the abovementioned PUFAs. Plants which are particularly suited to this purpose are oil crops, which comprise large amounts of lipid compounds, such as oilseed rape, canola, linseed, soyabeans, sunflowers, borage and evening primrose. However, other crop plants which comprise oils or lipids and fatty acids are also well suited, as mentioned in the extensive description of the present invention. Conventional breeding has given rise to a series of mutant plants which produce a spectrum of desirable lipids and fatty acids, cofactors and enzymes. However, the selection of new plant varieties with improved production of a particular molecule is a time-consuming and difficult procedure or is indeed impossible if the compound does not occur naturally in the plant in question, as in the case of polyunsaturated C_{18}-, C_{20}-fatty acids and C_{22}-fatty acids and those with longer carbon chains.

Owing to the positive properties of unsaturated fatty acids, there has been no lack of attempts in the past to make available these genes which are involved in the synthesis of fatty acids or triglycerides for the production, in various plants, of oils with a modified content of polyunsaturated fatty acids. However, it has been impossible as yet to produce longer-chain polyunsaturated C_{20}- and/or C_{22}-fatty acids such as EPA or ARA in

plants.

It was therefore an object to develop a method for the production of polyunsaturated fatty acid esters and/or free polyunsaturated fatty acids with at least three double bonds in the fatty acid molecule. This object was achieved by the method according to the invention for the production of compounds of the general formula I:



(I)

in transgenic plants with a content of at least 1% by weight based on the total fatty acids, which process comprises the following steps:

a) introducing, into a plant, at least one nucleic acid sequence which encodes a polypeptide with a Δ6-desaturase activity; and

b) introducing at least one second nucleic acid sequence which encodes a polypeptide with a Δ6-elongase activity; and,

c) if appropriate, introducing a third nucleic acid sequence which encodes a polypeptide with a Δ5-desaturase activity;

d) followed by growing and harvesting the plants; and

where the variables and substituents in the formula I have the following meanings:

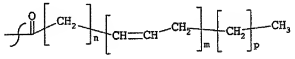
R^1 = -OH, coenzyme A (thioester), phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylserine, phosphatidylinositol, sphingolipid, glycoshingolipid or a radical of the following general formula II

(II)

R^2 = H, phosphatidylcholine-, phosphatidylethanolamine-, phosphatidylglycerol-, diphosphatidylglycerol-, phosphatidylserine-, phosphatidylinositol-, shingolipid-, glycoshingolipid-, glycoshingolipid- or saturated or unsaturated C_2–C_{24}–alkylcarbonyl-,

R^3 = H, saturated or unsaturated C_2–C_{24}–alkylcarbonyl-, or

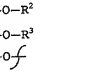
R^2 and R^3 independently of one another represent a radical of the general formula Ia



(Ia),

n = 3, 4 or 6, m = 3, 4 or 5 and p = 0 or 3, preferably n = 3, m = 4 or 5 and p = 0 or 3.

R^1 in the compounds of the formula I denotes -OH (hydroxyl-), acetyl-coenzyme A-, phosphatidylcholine-, phosphatidylethanolamine-, phoshatidylglycerol-, diphosphatidylglycerol-, phosphatidylserine-, phosphatidylinositol-, sphingolipid-, glycoshingolipid- or a radical of the following general formula II



(II)

The abovementioned radicals for R^1 are in each case bound to the compounds of the formula I in the form of esters or thioesters.

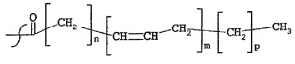
R^2 in the compounds of the formula II denotes hydrogen, phosphatidylcholine-, phosphatidylethanolamine-, phoshatidylglycerol-, diphosphatidylglycerol-, phosphatidylserine-, phosphatidylinositol-, shingolipid-, glycoshingolipid-, glycoshingolipid- or saturated or unsaturated C_2–C_{24}–alkylcarbonyl-.

Unsaturated or saturated C_2-C_{22}-alkylcarbonyl which may be mentioned are radicals such as ethylcarbonyl, n-propylcarbonyl, n-butylcarbonyl, n-pentylcarbonyl, n-hexylcarbonyl, n-heptylcarbonyl, n-octylcarbonyl, n-nonylcarbonyl,

n-decylcarbonyl, n-undecylcarbonyl, n-dodecylcarbonyl, n-tridecylcarbonyl, n-tetradecylcarbonyl, n-pentadecylcarbonyl, n-hexadecylcarbonyl, n-heptadecylcarbonyl, n-octadecylcarbonyl, n-nonadecylcarbonyl, n-eicosylcarbonyl, n-docosanylcarbonyl or n-tetracosanylcarbonyl, all of which may comprise one or more double bonds. Preferred are saturated or unsaturated C_{10}-C_{22}-alkylcarbonyl radicals such as n-decylcarbonyl, n-undecylcarbonyl, n-dodecylcarbonyl, n-tridecylcarbonyl, n-tetradecylcarbonyl, n-pentadecylcarbonyl, n-hexadecylcarbonyl, n-heptadecylcarbonyl, n-octadecylcarbonyl, n-nonadecylcarbonyl, n-eicosylcarbonyl, n-docosanylcarbonyl or n-tetracosanylcarbonyl, all of which comprise one or more double bonds. Especially preferred are saturated or unsaturated C_{10}–C_{22}–alkylcarbonyl radicals such as C_{10}–alkylcarbonyl, C_{11}–alkylcarbonyl, C_{12}–alkylcarbonyl, C_{13}–alkylcarbonyl, C_{14}–alkylcarbonyl, C_{16}–alkylcarbonyl, C_{18}–alkylcarbonyl, C_{20}–alkylcarbonyl, C_{22}–alkylcarbonyl or C_{24}–alkylcarbonyl radicals, all of which comprise one or more double bonds. Very especially preferred are saturated or unsaturated C_{16}–C_{22}–alkylcarbonyl radicals such as C_{16}–alkylcarbonyl, C_{18}–alkylcarbonyl, C_{20}–alkylcarbonyl or C_{22}–alkylcarbonyl radicals, all of which comprise one or more double bonds. Preferably, the abovementioned radicals comprise two, three, four or five double bonds. Especially preferably, the radicals comprise three, four or five double bonds. Very especially preferred are C_{18}–alkylcarbonyl radicals which comprise one, two, three or four double bonds and C_{20}–alkylcarbonyl radicals which comprise three, four or five double bonds. All of the abovementioned radicals are derived from the corresponding fatty acids.

R^3 denotes hydrogen or saturated or unsaturated C_2–C_{24}–alkylcarbonyl.

R^2 and R^3 in the compounds of the formula II independently of one another furthermore denote a radical of the general formula Ia



(Ia),

where n = 3, 4 or 6, m = 3, 4 or 5 and p = 0 or 3, preferably n = 3, m = 4 or 5 and p = 0 or 3.

The abovementioned radicals R^1, R^2 and R^3 may also have attached to them substituents such as hydroxyl or epoxy groups or else comprise triple bonds.

The nucleic acid sequences used in the method according to the invention are isolated nucleic acid sequences which encode polypeptides with Δ5-, Δ6-desaturase or Δ6-elongase activity.

The compounds of the formula I which are produced in this method advantageously comprise a mixture of differing radicals R^1, R^2 or R^3 which can be derived from differing glycerides. Moreover, the abovementioned radicals can be derived from different fatty acids such as short-chain fatty acids having 4 to 6 carbon atoms, medium-chain fatty acids having 8 to 12 carbon atoms or long-chain fatty acids having 14 to 24 carbon atoms; the long-chain fatty acids are preferred.

The method according to the invention advantageously gives fatty acid esters (= compounds of the formula I) with polyunsaturated C_{18}-, C_{20}- and/or C_{22}-fatty acid molecules with at least two double bonds in the fatty acid ester. Preferably, these fatty acid molecules comprise three, four or five double bonds and advantageously lead to the synthesis of γ-linolenic acid (= GLA, $C18:3^{\Delta 6,9,12}$), stearidonic acid (= SDA, $C18:4^{\Delta 6,9,12,15}$), dihomo-γ-linolenic acid (= DGLA, $20:3^{\Delta 8,11,14}$), eicosatetraenoic acid (= ETA, $C20:4^{\Delta 5,8,11,14}$), arachidonic acid (ARA), eicosapentaenoic acid (EPA) or their mixtures, preferably EPA and/or ARA.

The fatty acid esters with polyunsaturated C_{18}-, C_{20}- and/or C_{22}-fatty acid molecules can be isolated from the organisms which have been used for the production of the fatty acid esters in the form of an oil or lipid, for example in the form of compounds such as sphingolipids, phosphoglycerides, lipids, glycolipids such as glycosphingolipid, phospholipids such as phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatidylglycerol, phosphatidylinositol or diphosphatidylglycerol, monoacylglycerides, diacylglycerides, triacylglycerides or other fatty acid esters such as the acetyl-coenzyme A esters which comprise the polyunsaturated fatty acids having at least two, preferably three, double bonds. In addition to these esters, the polyunsaturated fatty acids are also present in the plants as free fatty acids or bound in other compounds. As a rule, the different abovementioned compounds (fatty acid esters and free fatty acids) are present in the plant in an approximate distribution of 80 to 90% by weight of triglycerides, 2 to 5% by weight of diglycerides, 5 to 10% by

weight of monoglycerides, 1 to 5% by weight of free fatty acids, 2 to 8% by weight of phospholipids, the total of the different compounds making 100% by weight.

When the compounds of the general formula I are produced in the method according to the invention, they are produced in a content of at least 1% by weight, advantageously at least 2% by weight, preferably at least 3% by weight, especially preferably at least 5% by weight, very especially preferably at least 10% by weight based on the total of the fatty acids in the transgenic plant. Since, in the method according to the invention, the starting compounds linoleic acid (C18:2) and/or linolenic acid (C18:3) undergo several reaction steps, the end products of the method, such as, for example, arachidonic acid (ARA) or eicosapentaenoic acid (EPA) are not obtained as pure products, but there are always minor amounts of the precursors still present in the end product. If both linoleic acid and linolenic acid are present in the original plant, the end products such as ARA and EPA are present as mixtures. The precursors should advantageously not amount to more than 20% by weight, preferably not more than 15% by weight, especially preferably not more than 10% by weight, very especially preferably not more than 5% by weight, based on the amount of the end product in question. Advantageously, the end products which are produced in the method according to the invention in a transgenic plant are only ARA or only EPA, either bound or as free acids (see compounds of the general formula I). If both compounds (ARA + EPA) are produced simultaneously, they are advantageously prduced in a ratio of at least 1:2 (EPA:ARA), advantageously at least 1:3, preferably 1:4, especially preferably 1:5.

Suitable organisms for the production in the method according to the invention are, in principle, all plants such as mosses, algae, dicots or monocots. It is advantageous to use, in the method according to the invention, organisms which belong to the oil-producing organisms, i.e. which are used for the production of oils, such as algae like Crypthecodinium, Phaeodactylum or plants, in particular plants, preferably oil crops, which comprise large amounts of lipid compounds, such as peanut, oilseed rape, canola, sunflower, safflower, poppy, mustard, hemp, castor-oil plant, olive, sesame, Calendula, Punica, evening primrose, verbascum, thistle, wild roses, hazelnut, almond, macadamia, avocado, bay, pumpkin/squash, linseed, soybean, pistachios, borage, trees (oil palm, coconut or walnut) or field crops such as maize, wheat, rye, oats, triticale, rice, barley, cotton, cassava, pepper, Tagetes, Solanaceae plants such as potato, tobacco, eggplant and tomato, Vicia species, pea, alfalfa

or bush plants (coffee, cacao, tea), Salix species and perennial grasses and fodder crops. Preferred plants according to the invention are oil crops such as peanut, oilseed rape, canola, sunflower, safflower, poa, mustard, hemp, castor-oil plants, olive, Calendula, Punica, evening primrose, pumpkin/squash, linseed, soybean, borage, trees (oil palm, coconut). Especially preferred are plants which are high in C10:2- and/or C18:3-fatty acid, such as sunflower, safflower, tobacco, verbascum, sesame, cotton, pumpkin/squash, poppy, evening primrose, walnut, linseed, hemp, thistle or safflower. Very especially preferred are plants such as safflower, sunflower, poppy, evening primrose, walnut, linseed or hemp.

Owing to the enzymatic activity of the nucleic acids used in the method according to the invention, which encode polypeptides with Δ5-, Δ6-desaturase or Δ6-elongase activity, different compounds of the formula I can be produced. Depending on the choice of the plant used for the method according to the invention, mixtures of the different compounds of the general formula I or individual compounds, such as EPA or ARA, can be produced in free or bound form. Depending on the fatty acid composition which prevails in the original plant (C18:2- or C18:3-fatty acids), this gives compounds of the general formula I which are derived from C18:2-fatty acids, such as GLA-, DGLA- or ARA-comprising compounds of the formula I, or compounds which are derived from C18:3-fatty acids, such as SDA-, ETA- or EPA-comprising compounds of the formula I. If linoleic acid (= LA, C18:$2^{\Delta 9,12}$) is the only unsaturated fatty acid present in the plant used for the method, only GLA, DGLA and ARA can be formed as products of the method, all of which can be present as free fatty acids or in bound form. If α-linolenic acid (= ALA, C18:$3^{\Delta 9,12,15}$) is the only unsaturated fatty acid present in the plant used in the method, for example such as in linseed, only SDA, ETA and EPA can be formed as products of the method, all of which can be present as free fatty acids or in bound form, as described above. By modifying the activity of the enzymes implicated in the synthesis (Δ5-, Δ6-desaturase and Δ6-elongase), or by introducing only the first two genes (Δ6-desaturase and Δ6-elongase) of the synthetic cascade, it is possible to produce in a targeted manner only individual products in the abovementioned plants (see Figure I). Due to the activity of the enzymes Δ6-desaturase and Δ6-elongase, GLA and DGLA, or SDA and ETA, respectively, form, depending on the original plant and the unsaturated fatty acid. DGLA or ETA, respectively, or mixtures of these are formed preferentially. If the enzyme Δ5-desaturase is additionally introduced into the plant, ARA or EPA are additionally formed. It is advantageous only to synthesize ARA or EPA or their mixtures, depending on the

fatty acid which is present in the plant and which acts as starting material for the synthesis. Since biosynthetic cascades are involved, the end products in question are not present in pure form in the plants. There are always minor amounts of the precursor compounds present in the end product. These minor amounts amount to less than 20% by weight, advantageously less than 15% by weight, especially advantageously less than 10% by weight, very especially advantageously less than 5, 4, 3, 2 or 1% by weight, based on the end product DGLA, ETA or their mixtures, or ARA, EPA or their mixtures, respectively.

For the purposes of the method according to the invention, transgenic plants are also understood as meaning plant cells, plant organs or intact plants which are grown for the production of compounds of the general formula I. Growing is understood as meaning for example culturing of the transgenic plant cells, plant tissue or plant organs on a nutrient medium or the intact plant on or in a substrate, for example in hydroponic culture or on an arable soil.

Nucleic acids which can be used in the method according to the invention are, in principle, all those which encode polypeptides with Δ5-, Δ6-desaturase- or Δ6-elongase activity. These nucleic acids are advantageously derived from plants such as algae, such as Isochrysis or Crypthecodinium, diatoms such as Phaeodactylum, mosses such as Physcomitrella, Ceratodon or higher plants such as the primulaceae, such as Aleuritia, Calendula stellata, Osteospermum spinescens or Osteospermum hyoseroides, microorganisms such as fungi, such as Aspergillus, Thraustochytrium, Phytophtora, Entomophthora, Mucor or Mortierella, yeasts or animals such as nematodes, such as Caenorhabditis, insects or humans. The Δ5-, Δ6-desaturase or Δ6-elongase genes are advantageously derived from fungi or from plants such as algae or mosses, preferably from plants.

It is advantageous to in the method according to the invention, a nucleic acid sequence selected from the group of the in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 or 31 or their derivative or homologs which encode polypeptides which retain the enzymatic activity. These sequences, individually or in combination, are cloned into expression constructs; these expression constructs are represented in the sequences SEQ ID NO: 33-37. These expression constructs make possible an optimal synthesis of the compounds of the general formula I produced in the method according to the invention.

In a preferred embodiment, the method furthermore comprises the step of obtaining a cell which comprises the nucleic acid sequences which are used in the method and which encode a Δ5- or Δ6-desaturase and a Δ6-elongase, where a cell is transformed with the nucleic acid sequence, a gene construct or a vector which bring about the expression of the Δ5-, Δ6-desaturase or Δ6-elongase nucleic acid, alone or in combination. In a further preferred embodiment, the method furthermore comprises the step of obtaining the fine chemical from the culture. The cell generated thus is advantageously a cell of an oil crop such as, for example, peanut, oilseed rape, canola, linseed, soybean, safflower, hemp, sunflowers or borage.

A transgenic plant is understood as meaning, for the purposes of the invention, that the nucleic acids used in the method are not at their natural locus in the genome of an organism; in this context, the nucleic acids can be expressed homologously or heterologously. However, transgenic also means that, while the nucleic acids according to the invention are at their natural locus in the genome of an organism, the sequence has been modified in comparison with the natural sequence and/or the regulatory sequences of the natural sequences have been modified. Preferably, transgenic is understood as meaning that the nucleic acids according to the invention are not expressed at their natural locus in the genome, that is to say that homologous or preferably heterologous expression of the nucleic acids takes place. Preferred transgenic plants are the oil crops.

Transgenic plants which comprise the compounds of the formula I which have been synthesized in the method according to the invention can be marketed directly without isolation of the compounds which have been synthesized. Plants are understood as meaning, in the method according to the invention, all plant parts, plant organs such as leaf, stem, root, tuber or seeds, or all of the plant. In this context, the seed comprises all parts of the seed such as the seed coats, epidermis cells and seed cells, endosperm or embyro tissue. However, the compounds produced in the method according to the invention can also be isolated from the plants in the form of their oils, fat, lipids and/or free fatty acids. Compounds of the formula I which have been produced by this method can be harvested by harvesting the organisms either from the culture in which they grow or from the field. This can be done by pressing or extracting the plant parts, preferably the plant seeds. In this context, the oils, fats, lipids and/or free fatty acids can be obtained by pressing by what is known as cold-beating or cold-pressing, without supplying heat. The plant parts, specifically the seeds, are

beforehand comminuted, steam-treated or toasted in order to facilitate their disruption. The seeds pretreated thus can subsequently be pressed or else extracted with solvents such as warm hexane. The solvent is subsequently removed. In this manner, more than 96% of the compounds produced in the method can be isolated. The resulting products are subsequently processed further, i.e. refined. Here, the plant mucilages and turbid matter are first. What is known as degumming can be performed enzymatically or, for example, chemico-physically by adding acid such as phosphoric acid. The free fatty acids are subsequently removed by treatment with a base, for example sodium hydroxide solution. The resulting product is washed thoroughly with water to remove the alkali remaining in the product, and dried. To remove the coloring matter which still remains in the product, the products are bleached, for example using bleaching earth or active charcoal. At the end, the product is deodorized, for example by using steam.

The PUFAs produced by this method are preferentially C_{18}- or C_{20-22}-fatty acid molecules having at least two double bonds in the fatty acid molecule, preferably three, four, in combination with a further elongases and a Δ4-desaturase five or six double bonds. These C_{18}- or C_{20-22}-fatty acid molecules can be isolated from the organism in the form of an oil, lipid or a free fatty acid. Suitable organisms are, for example, those which have been mentioned above. Preferred organisms are transgenic plants.

In a preferred embodiment, oils, lipids or fatty acids or fractions of these which have been produced by the above-described method are especially preferably oil, lipid or a fatty acid composition which comprise PUFAs or which originate from transgenic plants.

A further embodiment according to the invention is the use of the oil, lipid or the fatty acid composition in foods, feeds, cosmetics or pharmaceuticals.

The term "oil" or "fat" is understood as meaning a fatty acid mixture which comprises unsaturated, saturated, preferably esterified fatty acid(s). It is preferred that the oil or fat has a high content of unsaturated, unconjugated esterified fatty acid(s), in particular linoleic acid, γ-linolenic acid, dihomo-γ-linolenic acid, arachidonic acid, α-linolenic acid, stearidonic acid, eicosatetraenoic acid or eicosapentaenoic acid. The amount of unsaturated esterified fatty acids is preferably approximately 30%, with an amount of 50% being more preferred and an amount of 60%, 70%, 80% or more being even more preferred. For

identification purposes, it is possible, for example, to determine the amount of fatty acid by gas chromatography after converting the fatty acids into the methyl esters by means of transesterification. The oil or fat can comprise various other saturated or unsaturated fatty acids, for example calendulic acid, palmitic acid, stearic acid, oleic acid and the like. The amount of the various fatty acids in oil or fat can vary in particular as a function of the original plant.

The compounds of the formula I which are produced in the method and which comprise polyunsaturated fatty acids having at least two double bonds are sphingolipids, phosphoglycerides, lipids, glycolipids, phospholipids, monoacylglycerol, diacylglycerol, triacylglycerol or other fatty acid esters.

The polyunsaturated fatty acids which are present can be liberated from the compounds of the general formula I produced thus in the method according to the invention for example via treatment with alkali, for example aqueous KOH or NaOH, or acid hydrolysis, advantageously in the presence of an alcohol such as methanol or ethanol, or via enzymatic cleavage and isolated via, for example, phase separation and subsequent acidification with, for example, H_2SO_4. However, the fatty acids can also be liberated directly without the above-described processing.

After they have been introduced into plant cells or plants, the nucleic acids used in the method can either be located on a separate plasmid or integrated into the genome of the host cell. In the case of integration into the genome, the integration can be random or be effected by recombination in such a way that the native gene is replaced by the copy being introduced, whereby the production of the desired compound by the cell is modulated, or by using a gene in trans, so that the gene is linked operably with a functional expression unit which comprises at least one sequence which ensures the expression of a gene and at least one sequence which ensures the polyadenylation of a functionally transcribed gene. The nucleic acids are advantageously introduced into the plants via multiexpression cassettes or constructs for the multiparallel seed-specific expression of genes.

Mosses and algae are the only known plant systems which produce substantial amounts of polyunsaturated fatty acids such as arachidonic acid (ARA) and/or eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA). Mosses comprise PUFAs in membrane lipids, while algae, organisms which are related to algae and some fungi also accumulate substantial amounts of PUFAs in the triacylglycerol fraction. This is why nucleic acid molecules

which are isolated from such strains which also accumulate PUFAs in the triacylglycerol fraction are especially advantageously suitable for the method according to the invention and thus for the modification of the lipid and PUFA production system in a host, in particular plants, such as oil crops, for example oilseed rape, canola, linseed, hemp, soybean, sunflowers, borage. They can therefore be used advantageously in the method according to the invention.

It has been possible to date to demonstrate that a trienoic acid with C_{18} carbon chain can be produced with the aid of desaturases. These methods which are known from the literature claim the production of γ-linolenic acid. However, nobody has as yet been able to demonstrate the production very long-chain polyunsaturated fatty acids (with C_{20}- and longer carbon chain and of trienoic acids and higher unsaturated types) by modified plants alone.

To produce the longer-chain PUFAs according to the invention, the polyunsaturated C_{18}-fatty acids must first be desaturated by the enzymatic activity of a desaturase and subsequently elongated by at least two carbon atoms via an elongase. After one elongation cycle, this enzyme activity gives C_{20}-fatty acids, and after two or three elongation cycles C_{22}- or C_{24}-fatty acids. The activity of the desaturases and elongases used method according to the invention gives by preference C_{18}-, C_{20}- and/or C_{22}-fatty acids having at least two double bonds in the fatty acid molecule, by preference three, four or five double bonds, especially preferably C_{18}- and/or C_{20}-fatty acids with at least two double bonds in the fatty acid molecule, prefereably with three, four or five double bonds in the molecule. After a first desaturation and the elongation have taken place, further desaturation steps such as, for example, in Δ5-position, may take place. Especially preferred products of the process according to the invention are arachidonic acid and eicosapentaenoic acid. The C_{18}-fatty acids with at least two double bonds in the fatty acid can be elongated by the enzymatic activity according to the invention in the form of the free fatty acid or in the form of the esters, such as phospholipids, glycolipids, sphingolipids, phosphoglycerides, monoacylglycerol, diacylglycerol or triacylglycerol.

Using cloning vectors in plants and in the transformation of plants like those which are published and cited in: Plant Molecular Biology and Biotechnology (CRC Press, Boca Raton, Florida), Chapter 6/7, pp. 71-119 (1993); F.F. White, Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Vol. 1, Engineering and Utilization, Eds.: Kung and R. Wu, Academic

Press, 1993, 15-38; B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, Eds.: Kung and R. Wu, Academic Press (1993), 128-143; Potrykus, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42 (1991), 205-225)), the nucleic acids can be used for the recombinant modification of a broad spectrum of plants so that this plant becomes a better or more efficient producer of one or more lipid-derived products, such as PUFAs. This improved production or production efficiency of a lipid-derived product, such as PUFAs, can be brought about by a direct action of the manipulation or an indirect action of this manipulation.

A series of mechanisms exist by means of which the modification of a desaturase protein according to the invention can have a direct effect on the yield, production and/or production efficiency of a fine chemical from an oil crop plant or a microorganism, owing to a modified protein. The number or activity of the desaturase protein or desaturase gene and of gene combinations of desaturases and elongases can be increased, so that larger amounts of these compounds are produced de novo since the organisms lacked this activity and ability to biosynthesize them prior to introduction of the gene in question. This also applies analogously to the combination with further desaturases or elongases or further enzymes of the lipid metabolism. The use of various divergent sequences, i.e. sequences which differ at the DNA sequence level, may also be advantageous, or else the use of promoters for gene expression which makes possible a different temporal gene expression, for example as a function of the degree of maturity of the seed or oil-storing tissue.

The introduction of a desaturase and/or elongase gene, or several desaturase and elongase genes, into an organism, alone or in combination with other genes into a cell can not only increase the biosynthesis flux toward the end product, but also increase, or generate de novo, the corresponding triacylglycerol composition. Likewise, the number or activity of other genes which participate in the import of nutrients required for the biosynthesis of one or more fine chemicals (for example fatty acids, polar and neutral lipids) can be increased, so that the concentration of these precursors, cofactors or intermediates within the cells or within the storage compartment is increased, thus further increasing the ability of the cells to produce PUFAs as described hereinbelow. Fatty acids and lipids themselves are desirable as fine chemicals; by optimizing the activity or increasing the number of one or more desaturases and/or elongases which participate in the biosynthesis of these compounds, or by destroying the activity of one or more desaturases which

participate in the breakdown of these compounds, it can be possible to increase the yield, production and/or efficiency of the production of fatty acid and lipid molecules from plants.

The isolated nucleic acid molecules used in the process according to the invention encode proteins or parts of these, the proteins, or the individual protein or parts thereof, comprising an amino acid sequence with sufficient homology with an amino acid sequence of the sequence SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 or 32 so that the protein or the part thereof retains a desaturase or elongase activity. Preferably, the protein or the part thereof which is encoded by the nucleic acid molecule has its essential enzymatic activity and the capability of being implicated in the metabolism of compounds which are required for the synthesis of plant cell membranes or in the transport of molecules across these membranes. Advantageously, the protein encoded by the nucleic acid molecules is at least approximately 50%, preferably at least approximately 60% and more preferably at least approximately 70%, 80% or 90% and most preferably at least approximately 95%, 96%, 97%, 98%, 99% or more homologous to an amino acid sequence of the sequence SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 or 32. Preferably, the protein is a full-length protein which is essentially homologous in parts to a total amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 or 32 (which is the result of the open reading frame shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 or 31). For the purposes of the invention, homology and homologous are understood as meaning identity or identical.

The term essential enzymatic activity of the desaturases and the elongase used is understood as meaning that, in comparison with the proteins/enzymes encoded by the sequences with SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 or 31, they retain at least an enzymatic activity of at least 10%, preferably 20%, especially preferably 30% and very especially 40% and can thus be implicated in the metabolism of compounds which are required for the synthesis of fatty acids in a plant cell or in the transport of molecules across membranes, meaning desaturated C_{18}- or C_{20-22}- carbon chains with double bonds at at least two, advantageoulsy three, four or five positions.

Nucleic acids which can advantageously be used in the process originate from fungi or plants such as algae or mosses of the genera Physcomitrella, Thraustochytrium, Phytophtora, Ceratodon, Isochrysis, Aleurita, Muscarioides, Mortierella, Borago, Phaeodactylum, Crypthecodinium or from nematodes such as

Ceanorhabditis, specifically from the genera and species Physcomitrella patens, Phytophtora infestans, Ceratodon purpureus, Isochrysis galbana, Aleurita farinosa, Muscarioides viallii, Mortierella alpina, Borago officinalis, Phaeodactylum tricormutum or Ceanorhabditis elegans.

As an alternative, the isolated nucleotide sequences used can encode desaturases or elongases which hybridize, for example under stringent conditions, with a nucleotide sequence of the SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 or 31.

The nucleic acid sequences used in the process are advantageously introduced in an expression cassette which makes possible the expression of the nucleic acids in plants.

Advantageous expression cassettes are shown in SEQ ID NO: 33 to 37. Here, the nucleic acid sequences encoding the desaturases and/or the elongases are linked operably with one or more regulatory signals, advantageously for enhancing gene expression. These regulatory sequences are intended to make possible the specific expression of genes and of protein expression. Depending on the host organism, this may mean, for example, that the gene is expressed and/or overexpressed only after induction, or else that it is immediately expressed and/or overexpressed. For example, these regulatory sequences take the form of sequences to which inductors or repressors bind and thus regulate expression of the nucleic acid. In addition to these novel regulatory sequences, or instead of these sequences, the natural regulation of these sequences before the actual structural genes may still be present and, if appropriate, may have been genetically modified so that the natural regulation has been switched off and the expression of the genes enhanced. However, the expression cassette (= expression construct = gene construct) can also be simpler in construction, that is to say no additional regulatory signals have been inserted before the nucleic acid sequence or its derivatives, and the natural promoter together with its regulation has not been removed. Instead, the natural regulatory sequence has been mutated in such a way that regulation no longer takes place and/or gene expression is enhanced. These modified promoters can also be placed before the natural gene alone in the form of part-sequences (= promoter together with parts of the nucleic acid sequences according to the invention) to enhance the activity. Moreover, the gene construct can advantageously also comprise one or more enhancer sequences in operable linkage with the promoter, which make possible an enhanced expression of the nucleic acid sequence. Also, additional advantageous sequences,

such as further regulatory elements or terminators, may be inserted at the 3' terminus of the DNA sequences. The Δ5-desaturase/Δ6-desaturase and/or Δ6-elongase genes may be present in the expression cassette (= gene construct) in one or more copies. Advantageously, in each case only one copy of the genes is present in the expression cassette. This gene construct, or the gene constructs, can be expressed together in the host organism. In this context, the gene construct(s) can be inserted in one or more vectors and be present in the cell in free form or else be inserted in the genome. It is advantageous for the insertion of further genes in the host genome when the genes to be expressed are present together in one gene construct.

In this context, the regulatory sequences or factors can, as described above, preferably have a positive effect on the gene expression of the genes which have been introduced, thus enhancing it. Thus, the regulatory elements can advantageously be enhanced at transcriptional level by using strong transcription signals such as promoters and/or enhancers. In addition, however, an enhancement of translation is also possible, for example by improving the stability of the mRNA.

A further embodiment of the invention are one or more gene constructs which comprise one or more sequences which are defined by SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 or 31 and which encode polypeptides of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 or 32. The abovementioned desaturases introduce a double bond into the Δ5 or Δ6 position, the substrate having one, two, three or four double bonds. Elongase (Δ6-elongase) has an enzyme activity which elongates a fatty acid by at least two carbon atoms. The same applies to its homologs, derivatives or analogs which are linked operably with one or more regulatory signals, advantageously for enhancing gene expression.

Advantageous regulatory sequences for the novel process are present, for example, in promoters such as cos, tac, trp, tet, trp-tet, lpp, lac, lpp-lac, lacI^q, T7, T5, T3, gal, trc, ara, SP6, λ-P_R or λ-P_L promoter and are advantageously used in Gram-negative bacteria. Further advantageous regulatory sequences are present, for example, in the Gram-positive promoters amy and SPO2, in the yeast or fungal promoters ADC1, MFα, AC, P-60, CYC1, GAPDH, TEF, rp28, ADH or in the plant promoters CaMV/35S [Franck et al., Cell 21 (1980) 285-294], PRP1 [Ward et al., Plant. Mol. Biol. 22 (1993)], SSU, OCS, lib4, usp, STLS1, B33, nos or in the ubiquitin or phaseolin promoter. Also advantageous in this connection are inducible promoters such as the promoters described in EP-A-0

388 186 (benzylsulfonamide-inducible), Plant J. 2, 1992:397–404 (Gatz et al., tetracyclin-inducible), EP-A-0 335 528 (abscisic acid-inducible) or WO 93/21334 (ethanol- or cyclohexenol-inducible). Further useful plant promoters are the potato cytosolic FBPase promoter or ST-LSI promoter (Stockhaus et al., EMBO J. 8, 1989, 2445), the Glycine max phosphoribosyl-pyrophosphate amidotransferase promoter (Genbank Accession No. U87999) or the node-specific promoter described in EP-A-0 249 676. Especially advantageous promoters are promoters which make possible expression in tissues which are implicated in fatty acid biosynthesis. Very especially advantageous are seed-specific promoters, such as the USP promoter in accordance with the specification, but also other promoters such as the LeB4, DC3, phaseolin or napin promoter. Further especially advantageous promoters are seed-specific promoters which can be used for monocots or dicots and which are described in US 5,608,152 (oilseed rape napin promoter), WO 98/45461 (Arabidopsis oleosin promotor), US 5,504,200 (Phaseolus vulgaris phaseolin promoter), WO 91/13980 (Brassica Bce4 promoter) described by Baeumlein et al., Plant J., 2, 2, 1992:233–239 (LeB4 promoter from a legume), said promoters being useful in dicots. The following promoters are suitable for example in monocots: barley lpt-2 or lpt-1 promoter (WO 95/15389 and WO 95/23230), barley hordein promoter and other suitable promoters which are described in WO 99/16890.

In principle, it is possible to use all natural promoters with their regulatory sequences like those mentioned above for the novel process. It is likewise possible and advantageous to use synthetic promoters, in addition or alone, especially when they confer seed-specific expression, such as, for example, described in WO 99/16890.

In order to achieve a particularly high PUFA content in transgenic plants, the PUFA biosynthetic genes should advantageously be expressed in oil crops in a seed-specific manner. To this end, seed-specific promoters can be used, or those promoters which are active in the embryo and/or in the endosperm. In principle, seed-specific promoters can be isolated from both dicots and monocots. Advantageous preferred promoters are detailed hereinbelow: USP (= unknown seed protein) and vicilin (Vicia faba) [Bäumlein et al., Mol. Gen Genet., 1991, 225(3)], napin (oilseed rape) [US 5,608,152], Acyl-Carrier Protein (oilseed rape) [US 5,315,001 and WO 92/18634], oleosin (Arabidopsis thaliana) [WO 98/45461 and WO 93/20216], phaseolin (Phaseolus vulgaris) [US 5,504,200], Bce4 [WO 91/13980], legume B4 (LegB4 promoter) [Bäumlein et al., Plant J., 2, 2, 1992], Lpt2 and lpt1 (barley) [WO 95/15389 and WO95/23230], seed-specific

promoters from rice, maize and wheat [WO 99/16890], Amy32b, Amy 6-6 and aleurain [US 5,677,474], Bce4 (oilseed rape) [US 5,530,149], glycinin (soya) [EP 571 741], phosphoenolpyruvate carboxylase (soya) [JP 06/62870], ADR12-2 (soya) [WO 98/08962], isocitrate lyase (oilseed rape) [US 5,689,040] or β-amylase (barley) [EP 781 849].

Plant gene expression can also be facilitated via a chemically inducible promoter (see a review in Gatz 1997, Annu. Rev. Plant Physiol. Plant Mol. Biol., 48:89-108). Chemically inducible promoters are particularly suitable when it is desired that gene expression should take place in a time-specific manner. Examples of such promoters are a salicylic-acid-inducible promoter (WO 95/19443), a tetracyclin-inducible promoter (Gatz et al. (1992) Plant J. 2, 397-404) and an ethanol-inducible promoter.

To ensure the stable integration of the biosynthesis genes into the transgenic plant over a plurality of generations, each of the nucleic acids which encode Δ6-desaturase, Δ5-desaturase, or Δ6-elongase and which are used in the process should be expressed under the control of a separate promoter, preferably a promoter which differs from the other promoters, since repeating sequence motifs can lead to instability of the T-DNA, or to recombination events. In this context, the expression cassette is advantageously constructed in such a way that a promoter is followed by a suitable cleavage site, advantageously in a polylinker, for insertion of the nucleic acid to be expressed and, if appropriate, a terminator sequence is positioned behind the polylinker. This sequence is repeated several times, preferably three, four or five times, so that up to five genes can be combined in one construct and introduced into the transgenic plant in order to be expressed. Advantageously, the sequence is repeated up to three times (see sequence listing SEQ ID NO: 33 to 37). To express the nucleic acid sequences, the latter are inserted after the promoter via a suitable cleavage site, for example in the polylinker. Advantageously, each nucleic acid sequence has its own promoter and, if appropriate, its own terminator sequence. However, it is also possible to insert a plurality of nucleic acid sequences after a promoter and, if appropriate, before a terminator sequence. Here, the insertion site, or the sequence, of the inserted nucleic acids in the expression cassette is not of critical importance, that is to say a nucleic acid sequence can be inserted at the first or last position in the cassette without its expression being substantially influenced thereby. Advantageously, different promoters such as, for example, the USP, LegB4 or DC3 promoter, and different terminator sequences can be used in the expression

cassette. However, it is also possible to use only one type of promoter in the cassette, which, however, may lead to undesired recombination events.

As described above, the transcription of the genes which have been introduced should advantageously be terminated by suitable terminator sequences at the 3' end of the biosynthesis genes which have been introduced (after the stop codon). An example of a sequence which can be used in this context is the OCS1 terminator sequence. As is the case with the promoters, different terminator sequences should be used for each gene.

As described above, the gene construct can also comprise further genes to be introduced into the organisms. It is possible and advantageous to introduce into the host organisms, and to express, regulatory genes such as genes for inductors, repressors or enzymes which, owing to their enzyme activity, engage in the regulation of one or more genes of a biosynthesis pathway. These genes can be of heterologous or of homologous origin. Moreover, further biosynthesis genes of the fatty acid or lipid metabolism can advantageously be present in the nucleic acid construct, or gene construct; however, these genes can also be present on one or more further nucleic acid constructs. A biosynthesic gene of the fatty acid or lipid metabolism which is preferably chosen is a gene selected from the group acyl-CoA dehydrogenase(s), acyl-ACP [= acyl carrier protein] desaturase(s), acyl-ACP thioesterase(s), fatty acid acyltransferase(s), fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenases, lipoxygenases, triacylglycerol lipases, allene oxide synthases, hydroperoxide lyases or fatty acid elongase(s) or their combinations.

In this context, the abovementioned desaturases can be cloned into expression cassette according to the invention in combination with elongases and other desaturases and employed for the transformation of plants with the aid of Agrobacterium.

In this context, the regulatory sequences or factors can, as described above, have a positive effect on, preferably, the gene expression of the genes introduced, thus enhancing it. Thus, enhancement of the regulatory elements can advantageously take place at the transcriptional level by using strong transcription signals such as promoters and/or enhancers. In addition, however, enhancement of translation is also possible, for example by improving the stability of the mRNA. In principle, the expression

cassettes can be used directly for introduction into the plant, or else be introduced into a vectors.

These advantageous vectors, preferably expression vectors, comprise the nucleic acid which are used in the method and which encode Δ5- or Δ6-desatures or Δ6-elonagases, or a nucleic acid construct, which the nucleic acid used, alone or in combination with further biosynthetic genes of the fatty acid or lipid metabolism. As used in the present context, the term "vector" refers to a nucleic acid molecule which is capable of transporting another nucleic acid, to which it is bound. One type of vector is a "plasmid", which represents a circular double-stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, it being possible for additional DNA segments to be ligated in the viral genome. Certain vectors are capable of autonomous replication in a host cell in which they have been introduced (for example bacterial vectors with bacterial origin of replication). Other vectors are advantageously integrated in the genome of a host cell when being introduced into the host cell, whereby they replicate together with the host genome. Moreover, certain vectors are capable of governing the expression of genes with which they are operably linked. These vectors are referred to herein as "expression vectors". Usually, expression vectors which are suitable for DNA recombination techniques take the form of plasmids. In the present description, "plasmid" and "vector" can be used interchangeably since the plasmid is the most frequently used vector form. However the invention is also intended to comprise these other forms of expression vectors, such as viral vectors, which have similar functions. Furthermore, the term vector is also intended to comprise other vectors which are known to the skilled worker, such as phages, viruses such as SV40, CMV, TMV, transposons, IS elements, phasmids, phagemids, cosmids, linear or circular DNA.

The recombinant expression vectors which are advantageously used in the method comprise the nucleic acids described hereinbelow or the above-described gene construct in a form suitable for expressing these nucleic acids in a host cell, which means that the recombinant expression vectors comprise one or more regulatory sequences selected on the basis of the host cells to be used for the expression, which is linked operably with the nucleic acid sequence to be expressed. "Linked operably" in a recombinant expression vector means that the nucleotide sequence of interest is bound to the regulatory sequence(s) in such a way that the expression of the nucleotide sequence is possible and that they are bound with one another so that both sequences

fulfill the predicted function ascribed to the sequence (for example in an in-vitro transcription/translation system or in a host cell if the vector is introduced into the host cell). The term "regulatory sequence" is intended to comprise promoters, enhancers and other expression control elements (for example polyadenylation signals). These regulatory sequences are described for example in Goeddel: Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990), or see: Gruber and Crosby, in: Methods in Plant Molecular Biology and Biotechnolgy, CRC Press, Boca Raton, Florida, eds.: Glick and Thompson, chapter 7, 89-108, including the references therein. Regulatory sequences comprise those which govern the constitutive expression of a nucleotide sequence in many types of host cell and those which govern the direct expression of the nucleotide sequence only in specific host cells under specific conditions. The skilled worker knows that the design of the expression vector can depend on factors such as the choice of the host cell to be transformed, the expression level of the desired protein and the like.

The recombinant expression vectors used can be designed for expressing desaturases and elongases in prokaryotic or eukaryotic cells. This is advantageous since intermediate steps of vector construction are frequently performed in microorganisms for the sake of simplicity. For example, desaturase and/or elongase genes can be expressed in bacterial cells, insect cells (using baculovirus expression vectors), yeast cells and other fungal cells (see Romanos, M.A., et al. (1992) "Foreign gene expression in yeast: a review", Yeast 8:423-488; van den Hondel, C.A.M.J.J., et al. (1991) "Heterologous gene expression in filamentous fungi", in: More Gene Manipulations in Fungi, J.W. Bennet & L.L. Lasure, ed., pp. 396-428: Academic Press: San Diego; and van den Hondel, C.A.M.J.J., & Punt, P.J. (1991) "Gene transfer systems and vector development for filamentous fungi, in: Applied Molecular Genetics of Fungi, Peberdy, J.F., et al., ed., pp. 1-28, Cambridge University Press: Cambridge), Algen (Falciatore et al., 1999, Marine Biotechnology. 1, 3:239-251), ciliates of the types: Holotrichia, Peritrichia, Spirotrichia, Suctoria, Tetrahymena, Paramecium, Colpidium, Glaucoma, Platyophrya, Potomacus, Desaturaseudocohnilembus, Euplotes, Engelmaniella and Stylonychia, in particular the genus Stylonychia lemnae, using vectors by a transformation method as described in WO 98/01572, and preferably in cells of multi-celled plants (see Schmidt, R. and Willmitzer, L. (1988) "High efficiency *Agrobacterium tumefaciens*-mediated transformation of *Arabidopsis thaliana* leaf and cotyledon explants" Plant Cell Rep.:583-586; Plant Molecular Biology and Biotechnology, C Press, Boca Raton, Florida, chapter

6/7, pp.71-119 (1993); F.F. White, B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, vol. 1, Engineering and Utilization, ed.: Kung and R. Wu, Academic Press (1993), 128-43; Potrykus, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42 (1991), 205-225 (and references cited therein)). Suitable host cells are furthermore discussed in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). As an alternative, the recombinant expression vector can be transcribed and translated in vitro, for example using T7 promoter regulation sequences and T7 polymerase.

Protein expression in prokaryotes is usually performed with the aid of vectors which comprise constitutive or inducible promoters which govern the expression of fusion proteins or nonfusion proteins. Typical fusion expression vectors are, inter alia pGEX (Pharmacia Biotech Inc; Smith, D.B., and Johnson, K.S. (1988) Gene 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ), where glutathione S-transferase (GST), maltose-E-binding protein or protein A, respectively, is fused with the recombinant target protein.

Examples of suitable inducible nonfusion E. coli expression vectors are, inter alia, pTrc (Amann et al. (1988) Gene 69:301-315) and pET 11d (Studier et al., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, California (1990) 60-89). The target gene expression of the pTrc vector is based on the transcription of host RNA polymerase by a hybrid trp-lac fusion promoter. The target gene expression from the pET 11d vector is based on the transcription of a T7-gn10-lac fusion promoter, which is mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is provided by the host strains BL21 (DE3) or HMS174 (DE3) by a resident λ prophage which harbors a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

Other vectors which are suitable for use in prokaryotic organisms are known to the skilled worker; these vectors are, for example in E. coli, pLG338, pACYC184, the pBR series such as pBR322, the pUC series such as pUC18 or pUC19, the M113mp series, pKC30, pRep4, pHS1, pHS2, pPLc236, pMBL24, pLG200, pUR290, pIN-III^{113}-B1, λgt11 or pBdCI, in Streptomyces pIJ101, pIJ364, pIJ702 or pIJ361, in Bacillus pUB110, pC194 oder pBD214, in Corynebacterium pSA77 or pAJ667.

In a further embodiment, the expression vector is a yeast expression vector. Examples of vectors for expression in the yeast S. cerevisiae comprise pYeDesaturasec1 (Baldari et al.

(1987) Embo J. 6:229-234), pMFa (Kurjan and Herskowitz (1982) Cell 30:933-943), pJRY88 (Schultz et al. (1987) Gene 54:113-123) and pYES2 (Invitrogen Corporation, San Diego, CA). Vectors and methods for construction of vectors which are suitable for use in other fungi, such as the filamentous fungi, comprise those which are described in detail in: van den Hondel, C.A.M.J.J., & Punt, P.J. (1991) "Gene transfer systems and vector development for filamentous fungi, in: Applied Molecular Genetics of fungi, J.F. Peberdy et al., ed., pp. 1-28, Cambridge University Press: Cambridge, or in: More Gene Manipulations in Fungi [J.W. Bennet & L.L. Lasure, ed., pp. 396-428: Academic Press: San Diego]. Further suitable yeast vectors are, for example, pAG-1, YEp6, YEp13 or pEMBLYe23.

As an alternative, the desaturases and/or elongases can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors which are available for expressing proteins in cultured insect cells (for example Sf9 cells) comprise the pAc series (Smith et al. (1983) Mol. Cell Biol.. 3:2156-2165) and the pVL series (Lucklow and Summers (1989) Virology 170:31-39).

The abovementioned vectors offer only a small overview over suitable vectors which are possible. Further plasmids are known to the skilled worker and are described, for example, in: Cloning Vectors (ed. Pouwels, P.H., et al., Elsevier, Amsterdam-New York-Oxford, 1985, ISBN 0 444 904018). Further suitable expression systems for prokaryotic and eukaryotic cells, see in the chapters 16 and 17 of Sambrook, J., Fritsch, E.F., and Maniatis, T., Molecular Cloning: A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

In a further embodiment of the process, the desaturases and/or elongases can be expressed in single-cell plant cells (such as algae), see Falciatore et al., 1999, Marine Biotechnology 1 (3):239-251 and references cited therein, and plant cells from higher plants (for example spermatophytes such as crops). Examples of plant expression vectors comprise those which are described in detail in: Becker, D., Kemper, E., Schell, J., and Masterson, R. (1992) "New plant binary vectors with selectable markers located proximal to the left border", Plant Mol. Biol. 20:1195-1197; and Bevan, M.W. (1984) "Binary Agrobacterium vectors for plant transformation", Nucl. Acids Res. 12:8711-8721; Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, vol. 1, Engineering and Utilization, ed.: Kung and R. Wu, Academic Press, 1993, pp. 15-38.

A plant expression cassette preferably comprises regulatory sequences which are capable of governing the gene expression in plant cells and which are linked operably so that each sequence can fulfill its function, such as transcriptional termination, for example polyadenylation signals. Preferred polyadenylation signals are those which originate from Agrobacterium tumefaciens T-DNA, such as the gene 3 of the Ti plasmid pTiACH5, which is known as octopine synthase (Gielen et al., EMBO J. 3 (1984) 835ff.) or functional equivalents thereof, but all other terminators which are functionally active in plants are also suitable.

Since plant gene expression is very often not limited to the transcriptional levels, a plant expression cassette preferably comprises other operably linked sequences such as translation enhancers, for example the overdrive sequence which comprises the 5'-untranslated leader sequence from tobacco mosaic virus, which increases the protein/RNA ratio (Gallie et al., 1987, Nucl. Acids Research 15:8693-8711).

As described above, plant gene expression must be linked operably with a suitable promoter which performs gene expression with the correct timing or in a cell- or tissue-specific manner. Utilizable promoters are constitutive promoters (Benfey et al., EMBO J. 8 (1989) 2195-2202) such as those which are derived from plant viruses, such as 35S CAMV (Franck et al., Cell 21 (1980) 285-294), 19S CaMV (see also US 5352605 and WO 84/02913) or plant promoters such as the Rubisco small subunit, which is described in US 4,962,028.

Other sequences which are preferred for the use for operable linkage in plant gene expression cassettes are targeting sequences, which are required for targeting the gene product into its relevant cell compartment (for a review see Kermode, Crit. Rev. Plant Sci. 15, 4 (1996) 285-423 and references cited therein), for example into the vacuole, the nucleus, all types of plastids, such as amyloplasts, chloroplasts, chromoplasts, the extracellular space, the mitochondria, the endoplasmic reticulum, oil bodies, peroxisomes and other plant cell compartments.

Plant gene expression can also be facilitated as described above via a chemically inducible promoter (for a review see Gatz 1997, Annu. Rev. Plant Physiol. Plant Mol. Biol., 48:89-108). Chemically inducible promoters are suitable in particular when it is desired that gene expression is clock-specific. Examples of such promoters are a salicylic acid-inducible promoter (WO

95/19443), a tetracyclin-inducible promoter (Gatz et al. (1992) Plant J. 2, 397-404) and an ethanol-inducible promoter.

Other promoters which are suitable are promoters which respond to biotic or abiotic stress conditions, for example the pathogen-induced PRP1 gene promoter (Ward et al., Plant. Mol. Biol. 22 (1993) 361-366), the heat-inducible tomato hsp80 promoter (US 5,187,267), the chill-inducible potato alpha-amylase promoter (WO 96/12814) or the wound-inducible pinII promoter (EP-A-0 375 091).

Preferred promoters are in particular those which bring about the expression of genes in tissues and organs in which lipid and oil biosynthesis takes place, in seed cells, such as cells of the endosperm and of the developing embryo. Suitable promoters are the oilseed rape napin gene promoter (US 5,608,152), the Vicia faba USP promoter (Baeumlein et al., Mol Gen Genet, 1991, 225 (3):459-67), the Arabidopsis oleosin promoter (WO 98/45461), the Phaseolus vulgaris phaseolin promoter (US 5,504,200), the Brassica Bce4 promoter (WO 91/13980) or the legumin B4 promoter (LeB4; Baeumlein et al., 1992, Plant Journal, 2 (2):233-9), and promoters which bring about the seed-specific expression in monocots such as maize, barley, wheat, rye, rice and the like. Suitable promoters which should be taken into consideration are the barley lpt2 or lpt1 gene promoter (WO 95/15389 and WO 95/23230), or those described in WO 99/16890 (promoters from the barley hordein gene, the rice glutelin gene, the rice oryzin gene, the rice prolamin gene, the wheat gliadin gene, the wheat glutelin gene, the maize zein gene, the oat glutelin gene, the sorghum kasirin gene, the rye secalin gene).

In particular, it may be desired to bring about the multiparallel expression of the desaturases and/or elongases used in the method alone or in combination with other desaturases or elongases. Such expression cassettes can be introduced via the simultaneous transformation of a plurality of individual expression constructs or, preferably, by combining a plurality of expression cassettes on one construct. Also, it is possible to transform a plurality of vectors with in each case a plurality of expression cassettes and to transfer them to the host cell.

Promoters which are likewise especially suitable are those which bring about the plastid-specific expression since plastids are the compartment in which the precursors and some end products of lipid biosynthesis are synthetized. Suitable promoters such as the viral RNA polymerase promoter are described in WO 95/16783

and WO 97/06250, and the Arabidopsis clpP promoter, described in WO 99/46394.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. The terms "transformation" and "transfection", conjugation and transduction, as used in the present context, are meant to comprise a multiplicity of methods known in the art for introducing foreign nucleic acid (for example DNA) into a host cell, including calcium phosphate or calcium chloride coprecipitation, DEAE-dextran-mediated transfection, lipofection, natural competence, chemically mediated transfer, electroporation or particle bombardment. Suitable methods for the transformation or transfection of host cells, including plant cells, can be found in Sambrook et al. (Molecular Cloning: A Laboratory Manual., 2nd edition., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) and other laboratory handbooks such as Methods in Molecular Biology, 1995, vol. 44, Agrobacterium protocols, ed.: Gartland and Davey, Humana Press, Totowa, New Jersey.

Host cells which are suitable in principle for taking up the nucleic acid according to the invention, the gene product according to the invention or the vector according to the invention are all prokaryotic or eukaryotic organisms. The host organisms which are advantageously used are organisms such as bacteria, fungi, yeasts or plant cells, preferably plants or parts thereof. Fungi, yeasts or plants are used by preference; especially preferably plants, very especially preferably plants such as oil crops which comprise large amounts of lipid compounds, such as oilseed rape, evening primrose, hemp, thistle, peanut, canola, linseed, soya, safflower, sunflower, borage or plants such as maize, wheat, rye, oats, triticale, rice, barley, cotton, cassava, pepper, Tagetes, Solanaceae plants such as potato, tobacco, eggplant and tomato, Vicia species, pea, alfalfa, bushy plants (coffee, cocoa, tea), Salix species, trees (oil palm, coconut) and perennial grasses and fodder crops. Especially preferred plants according to the invention are oil crops such as soya, peanut, oilseed rape, canola, linseed, hemp, evening primrose, sunflower, safflower, trees (oil palm, coconut).

Nucleic acid sequences which are advantageously used in the process according to the invention are those which encode polypeptides with a Δ6-desaturase activity, Δ6-elongase activity or Δ5-desaturase activity, selected from the group consisting of:

a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29 or SEQ ID NO: 31,

b) nucleic acid sequences which, owing to the degeneracy of the genetic code, are obtained by back translation of the amino acid sequences shown in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30 or SEQ ID NO: 32,

c) derivatives of the nucleic acid sequences shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29 or SEQ ID NO: 31 which encode polypeptides with the amino acid sequences shown in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30 or SEQ ID NO: 32 and which have at least 50% homology at the amino acid level, without the enzymatic activity of the polypeptides being substantially reduced.

The abovementioned nucleic acid according to the invention originates from organisms such as animals, ciliates, fungi, plants such as algae or dinoflagellates which are capable of synthesizing PUFAs.

The term "nucleic acid (molecule)" as used in the present context also comprises the untranslated sequence located at the 3' and at the 5' end of the coding gene region: at least 500, preferably 200, especially preferably 100 nucleotides of the sequence upstream of the 5' terminus of the coding region and at least 100, preferably 50, especially preferably 20 nucleotides of the sequence downstream of the 3' end of the coding gene region. An "isolated" nucleic acid molecule is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. An "isolated" nucleic acid preferably has no sequences which naturally flank the nucleic acid in the genomic DNA of the organism from which the nucleic acid originates (for example sequences which are present at the 5' and 3' ends of the nucleic acid). In different embodiments, the isolated desaturase

or elongase nucleic acid molecule may comprise, for example less than approximately 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in the genomic DNA of the cell from which the nucleic acid originates.

The nucleic acid molecules used in the process, for example a nucleic acid molecule with a nucleotide sequence of the SEQ ID NO: 1 or a part thereof, can be isolated using molecular-biological standard techniques and the sequence information provided herein. Also, for example a homologous sequence or homologous, conserved sequence regions at the DNA or amino acid level can be identified with the aid of comparative algorithms. They can be used as hybridization probe and standard hybridization techniques (as described, for example, in Sambrook et al., Molecular Cloning: A Laboratory Manual. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) for the isolation of further nucleic acid sequences which are useful in the process. Moreover, a nucleic acid molecule comprising a complete sequence of the SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 or 31 or a part thereof can be isolated by polymerase chain reaction, where oligonucleotide primers, which are used on the basis of this sequence or parts thereof (for example, it is possible to isolate a nucleic acid molecule comprising the complete sequence or a part thereof by means of polymerase chain reaction using oligonucleotide primers which have been generated on the basis of the same sequence). For example, mRNA can be isolated from cells (for example by means of the guanidinium thiocyanate extraction method of Chirgwin et al. (1979) Biochemistry 18:5294-5299) and cDNA can be generated by means of reverse transcriptase (for example Moloney MLV Reverse Transcriptase, available from Gibco/BRL, Bethesda, MD, or AMV Reverse Transcriptase, available from Seikagaku America, Inc., St.Petersburg, FL). Synthetic oligonucleotide primers for amplification by means of polymerase chain reaction can be generated based on one of the sequences shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 or 31 and that of Figure 5a, or with the aid of the amino acid sequences shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 or 32. A nucleic acid according to the invention can be amplified in accordance with standard PCR amplification techniques using cDNA or, alternatively, genomic DNA as template and suitable oligonucleotide primers. The nucleic acid amplified thus can be cloned into a suitable vector and characterized by means of DNA sequence analysis. Oligonucleotides which correspond to a desaturase nucleotide sequence can be generated by means of

synthetic standard methods, for example using an automatic DNA synthesizer.

Homologs of the desaturase or elongase nucleic acid sequences used, with sequence SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 or 31, means for example allelic variants with at least approximately 50 to 60%, preferably at least approximately 60 to 70%, more preferably at least approximately 70 to 80%, 80 to 90% or 90 to 95% and even more preferably at least approximately 95%, 96%, 97%, 98%, 99% or more homology with one of the nucleotide sequences shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 or 31 or their homologs, derivatives or analogs, or parts of these. Moreover, isolated nucleic acid molecules of a nucleotide sequence which hybridize with one of the nucleotide sequences shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 or 31 or part thereof, hybridize for example under stringent conditions. Allelic variants comprise in particular functional variants which can be obtained by deletion, insertion or substitution of nucleotides from/into the sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 or 31, it being intended, however, that the enzyme activity of the resulting synthesized proteins is advantageously retained for the insertion of one or more genes. Proteins which retain the enzymatic activity of the desaturase or elongase, i.e. whose activity is essentially not reduced, means proteins with at least 10%, preferably 20%, especially preferably 30%, very especially preferably 40% of the original enzyme activity in comparison with the protein encoded by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 or 32.

Homologs of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 or 31 mean for example also bacterial, fungal and plant homologs, truncated sequences, single-stranded DNA or RNA of the coding and noncoding DNA sequence.

Homologs of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 or 31 also means derivatives such as, for example, promoter variants. The promoters upstream of the abovementioned nucleotide sequences can be modified by one or more nucleotide substitutions, insertion(s) and/or deletion(s) without, however, interfering with the functionality or activity of the promoters. Moreover, it is possible to increase the activity of the promoters by modifying their sequence or to replace them completely by more active promoters, including promoters from heterologous organisms.

The abovementioned nucleic acids and protein molecules with desaturase or elongase activity which are involved in the metabolism of lipids and fatty acids, PUFA cofactors and enzymes or in the transport of lipophilic compounds across membranes are used in the process according to the invention for the modulation of the production of compounds of the general formula I in transgenic plants such as maize, wheat, rye, oats, triticale, rice, barley, soybean, peanut, cotton, Linum species such as linseed or flax, Brassica species such as oilseed rape, canola and turnip rape, pepper, sunflower, borage, evening primrose and Tagetes, Solanaceae plants such as potato, tobacco, eggplant and tomato, Vicia species, pea, cassava, alfalfa, bushy plants (coffee, cocoa, tea), Salix species, trees (oil palm, coconut) and perennial grasses and fodder crops, either directly (for example when the overexpression or optimization of a fatty acid biosynthesis protein has a direct effect on the yield, production and/or production efficiency of the fatty acid from modified organisms) and/or can have an indirect effect which nevertheless leads to an increase in the yield, production and/or production efficiency of a desired compound or a decrease in undesired compounds (for example when the modulation of the metabolism of lipids and fatty acids, cofactors and enzymes leads to modifications of the yield, production and/or production efficiency or the composition of the desired compounds within the cells, which, in turn, may have an effect on the production of one or more fatty acids).

The combination of different precursor molecules and biosynthetic enzymes results in the production of different fatty acid molecules, which has a decisive effect on lipid composition. Since polyunsaturated fatty acids (= PUFAs) are not simply incorporated into triacylglycerol, but also into membrane lipids.

Lipid synthesis can be divided into two sections: the synthesis of fatty acids and their binding to sn-glycerol-3-phosphate, and the addition or modification of a polar head group. Conventional lipids which are used in membranes comprise phospholipids, glycolipids, sphingolipids and phosphoglycerides. Fatty acid synthesis starts with the conversion of acetyl-CoA into malonyl-CoA by the enzyme acetyl-CoA carboxylase or into acetyl-ACP by the enzyme acetyl transacylase. After a condensation reaction, these two product molecules together form acetoacetyl-ACP, which is converted by a series of condensation, reduction and dehydratization reactions so that a saturated fatty acid molecule with the desired chain length is obtained. The production of the unsaturated fatty acids from these molecules is catalyzed by specific desaturases, either aerobically by means of

molecular oxygen or anaerobically (as regards the fatty acid synthesis in microorganisms, see F.C. Neidhardt et al. (1996) E. coli and Salmonella. ASM Press: Washington, D.C., pp. 612-636 and references cited therein; Lengeler et al. (ed.) (1999) Biology of Procaryotes. Thieme: Stuttgart, New York, and references therein, and Magnuson, K., et al. (1993) Microbiological Reviews 57:522-542 and the references therein).

Examples of precursors for PUFA biosynthesis are oleic acid, linoleic acid and linolenic acid. These C_{18}-carbon fatty acids must be elongated to C_{20} and C_{22} to obtain fatty acids of the eicosa and docosa chain type. With the aid of the desaturases used in the process, such as Δ5- and Δ6-desaturase and Δ6-elongase, it is possible to obtain arachidonic acid and eicosapentaenoic acid and various other long-chain PUFAs, to extract them and to use them for various purposes in applications in foodstuffs, feeding stuffs, cosmetics or pharmacology. Using the abovementioned enzymes, it is possible to produce preferably C_{18} + C_{20} fatty acids with at least two, three, four or five double bonds in the fatty acid molecule, preferably C_{20}-fatty acids with advantageously three, four or five double bonds in the fatty acid molecule. Desaturation can take place before or after elongation of the fatty acid in question. This is why the products of desaturase activities and the further desaturation and elongation which are possible give rise to preferred PUFAs with a higher degree of desaturation, including a further elongation from C_{20} to C_{22}-fatty acids, to give fatty acids such as γ-linolenic acid, dihomo-γ-linolenic acid, arachidonic acid, stearidonic acid, eicosatetraenoic acid or eicosapentaenoic acid. Substrates in the process according to the invention are, for example, linoleic acid, γ-linolenic acid, α-linolenic acid, dihomo-γ-linolenic acid, eicosatetraenoic acid or stearidonic acid. Preferred substrates are linoleic acid, γ-linolenic acid and/or α-linolenic acid, dihomo-γ-linolenic acid or arachidonic acid, eicosatetraenoic acid or eicosapentaenoic acid, respectively. The C_{18}- or C_{20}-fatty acids with at least two double bonds in the fatty acid are obtained in the process according to the invention in the form of the free fatty acid or in the form of its esters (see formula I), for example in the form of its glycerides.

The term "glyceride" is understood as meaning a glycerol which is esterified with one, two or three carboxylic acid residues (mono-, di- or triglyceride). "Glyceride" is also understood as being a mixture of various glycerides. The glyceride, or glyceride mixture, may comprise further additions, for example

free fatty acids, antioxidants, proteins, carbohydrates, vitamins and/or other substances.

A "glyceride" for the purposes of the process according to the invention is furthermore understood as meaning glycerol-derived derivatives. These include, in addition to the above-described fatty acid glycerides, glycerophospholipids and glycerolglycolipids. Preferred examples which may be mentioned in this context are the glycerophospholipids such as lecithin (phosphatidylcholine), cardiolipin, phosphatidylglycerol, phosphatidylserine and alkylacylglycerophospholipids.

Furthermore, fatty acids must subsequently be translocated to various sites of modification and incorporated into the triacylglycerol storage lipid. A further important step in lipid synthesis is the transfer of fatty acids on the polar head groups, for example by the enzyme glycerol fatty acid acyltransferase (see Frentzen, 1998, Lipid, 100(4-5):161-166).

Publications on plant fatty acid biosynthesis, desaturation, the lipid metabolism and membrane transport of lipidic compounds, beta-oxidation, fatty acid modification and cofactors, triacylglycerol storage and triacylglycerol assembly including the references cited therein, see the following papers: Kinney, 1997, Genetic Engeneering, ed.: JK Setlow, 19:149-166; Ohlrogge and Browse, 1995, Plant Cell 7:957-970; Shanklin and Cahoon, 1998, Annu. Rev. Plant Physiol. Plant Mol. Biol. 49:611-641; Voelker, 1996, Genetic Engeneering, ed.: JK Setlow, 18:111-13; Gerhardt, 1992, Prog. Lipid R. 31:397-417; Gühnemann-Schäfer & Kindl, 1995, Biochim. Biophys Acta 1256:181-186; Kunau et al., 1995, Prog. Lipid Res. 34:267-342; Stymne et al., 1993, in: Biochemistry and Molecular Biology of Membrane and Storage Lipids of Plants, ed.: Murata and Somerville, Rockville, American Society of Plant Physiologists, 150-158, Murphy & Ross 1998, Plant Journal. 13(1):1-16.

The PUFAs produced in the process comprise a group of molecules which higher animals are no longer capable of synthesising and must therefore take up, or which higher animals can no longer synthesize themselves in sufficient amounts and must thus additionally take them up, although they are synthesized readily by other organisms such as bacteria; for example, cats are no longer capable of synthesizing arachidonic acid.

For the purposes of the invention, the terms "desaturase or elongase" or "desaturase or elongase polypeptide" comprises proteins which are implicated in the desaturation and elongation

of fatty acids, and their homologs, derivatives or analogs. The terms desaturase or elongase nucleic acid sequence(s) comprise nucleic acid sequences which encode a desaturase or elongase and in which a part can be a coding region and likewise corresponding 5'- and 3'-untranslated sequence regions. The terms production or producitivity are known in the art and comprise the concentration of the fermentation product (compound of the formula I) which is formed within a specified period of time and a specified fermentation volume (for example kg of product per hour per liter). The term production efficiency comprises the time span required for obtaining a specific amount of product (for example the time required by the cell for establishing a certain throughput rate of a fine chemical). The term yield or product/carbon yield is known in the art and comprises the efficiency with which the carbon source is converted into the product (i.e. the fine chemical). This is usually expressed as, for example, kg of product per kg of carbon source. Increasing the yield or production of the compound results in increasing the amount of resulting molecules or the suitable resulting molecules of this compound in a certain amount of culture over a specified period. The terms biosynthesis or biosynthetic pathway are known in the art and comprise the synthesis of a compound, preferably an organic compound, by a cell starting from intermediates, for example in a multi-step process which is strongly regulated. The terms catabolism or catabolic pathway are known in the art and comprise the cleavage of a compound, preferably an organic compound, by a cell to give catabolites (in more general germs, smaller or less complex molecules), for example in a multi-step process which is strongly regulated. The term metabolism is known in the art and comprises the totality of the biochemical reactions which take place in an organism. The metabolism of a certain compound (for example the metabolism of a fatty acid) thus comprises the totality of the biosynthetic pathways, modified pathways and catabolic pathways of this compound in the cell which relate to this compound.

In a further embodiment, derivatives of the nucleic acid molecule according to the invention encode proteins with at least 50%, advantageously approximately 50 to 60%, preferably at least approximately 60 to 70% and more preferably at least approximately 70 to 80%, 80 to 90%, 90 to 95% and most preferably at least approximately 96%, 97%, 98%, 99% or more homology (= identity) with a complete amino acid sequence of the SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 or 32. The homology of the amino acid sequence can be determined over the entire sequence region using the program PileUp (J. Mol. Evolution., 25, 351-360, 1987, Higgins et al., CABIOS, 5,

1989:151–153) or BESTFIT or GAP (Henikoff, S. and Henikoff, J. G. (1992). Amino acid substitution matrices from protein blocks. Proc. Natl. Acad. Sci. USA 89: 10915-10919.)

Moreover, the invention comprises nucleic acid molecules which differ from one of the nucleotide sequences shown in SEQ ID NO: 1, 3, 5 or 11 (and parts thereof) as the result of the degeneracy of the genetic code and which thus encode the same desaturase as the desaturase which is encoded by the nucleotide sequences shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 or 31.

In addition to the desaturase nucleotide sequences shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 or 31, the skilled worker will recognize that DNA sequence polymorphisms which result in modifications in the amino acid sequences of the desaturases or elongases may exist within a population. These genetic polymorphisms in the desaturase or elongase gene may exist between individuals within a population as the result of natural variation. These natural variants usually bring about a variance of from 1 to 5% in the nucleotide sequence of the desaturase or elongase gene. All and sundry of these nucleotide variations and resulting amino acid polymorphisms in the enzyme desaturase or elongase which are the result of natural variation and which do not modify the functional activity of desaturases or elongases are also intended to fall under the scope of the invention.

Nucleic acid molecules which are advantageous for the process according to the invention can be isolated on the basis of their homology with the desaturase or elongase nucleic acids disclosed herein using the sequences or part thereof as hybridization probe, following standard hybridization techniques under stringent hybridization conditions. In this context, it is possible for example to use isolated nucleic acid molecules which are at least 15 nucleotides in length and which hybridize under stringent conditions with the nucleic acid molecules which comprise a nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 or 31. It is also possible to use nucleic acids with at least 25, 50, 100, 250 or more nucleotides. The term "hybridizes under stringent conditions" as used in the present context is understood as describing hybridization and wash conditions under which nucleotide sequences with at least 60% homology with one another usually remain hybridized with one another. The conditions are preferably such that sequences which are at least approximately 65%, more preferably at least approximably 70% and even more preferably at

least approximately 75% or more homologous with one another usually remain hybridized with one another. These stringent conditions are known to the skilled worker and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N. Y. (1989), 6.3.1-6.3.6. A preferred nonlimiting example of stringent hybridization conditions is hybridization in 6 x sodium chloride/sodium citrate (SSC) at approximately 45°C, followed by one or more wash steps in 0.2 x SSC, 0.1% SDS at 50 to 65°C. The skilled worker knows that these hybridization conditions differ depending on the type of the nucleic acid and, for example when organic solvents are used, with regard to the temperature and concentration of the buffer. For example, under "standard hybridization conditions" the temperature differs depending on the type of nucleic acid between 42°C and 58°C in aqueous buffer with a concentration of 0.1 to 5 x SSC (pH 7.2). If organic solvent is present in the abovementioned buffer, for example 50% formamide, the temperature under standard conditions is approximately 42°C. The hybridization conditions for DNA:DNA hybrids preferably are for example 0.1 x SSC and 20°C to 45°C, preferably between 30°C and 45°C. The hybridization conditions for DNA:RNA hybrids preferably are for example 0.1 x SSC and 30°C to 55°C, preferably between 45°C and 55°C. The abovementioned hybridization temperatures are determined for example for a nucleic acid with a length of approximately 100 bp (= base pairs) and a G + C content of 50% in the absence of formamide. The skilled worker knows how to identify the hybridization conditions required with the aid of textbooks, such as the one mentioned above, or the following textbooks: Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989; Hames and Higgins (ed.) 1985, "Nucleic Acids Hybridization: A Practical Approach", IRL Press at Oxford University Press, Oxford; Brown (ed.) 1991, "Essential Molecular Biology: A Practical Approach", IRL Press at Oxford University Press, Oxford.

To determine the percentage homology (= identity) of two amino acid sequences (for example of the sequences of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 or 32) or of two nucleic acids (for example one of the sequences of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 or 31), the sequences are written underneath each other to provide an optimal comparison (for example, gaps may be introduced into the sequence of a protein or a nucleic acid in order to generate an optimal alignment with the other protein or the other nucleic acid). The amino residues of nucleotides at the corresponding amino acid positions or nucleotide positions are then compared. If a position in a sequence is occupied by the same amino acid residue or the same nucleotide as the corresponding position in the other

sequence, the molecules are homologous at this position (i.e. amino acid or nucleic acid "homology" as used in the present context corresponds to amino acid or nucleic acid "identity"). The percentage homology between the two sequences is a function of the number of identical positions which the sequences share (i.e. percent homology = number of identical positions/total number of positions x 100). The terms homology and identity are thus to be regarded as synonymous.

An isolated nucleic acid molecule which encodes a desaturase or elongase which is homologous to a protein sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 or 32 can be generated by introducing one or more nucleotide substitutions, additions or deletions into a nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 or 31 so that one or more amino acid substitutions, additions or deletions are introduced into the protein which is encoded. Mutations can be introduced into one of the sequences of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 or 31 by standard techniques such as site-specific mutagenesis and PCR-mediated mutagenesis. It is preferred to generate conservative amino acid substitutions at one or more of the predicted nonessential amino acid residues. In a "conservative amino acid substitution", the amino acid residue is substituted by an amino acid residue with a similar side chain. Families of amino acid residues with similar side chains have been defined in the art. These families comprise amino acids with basic side chains (for example lysine, arginine, histidine), acidic side chains (for example aspartic acid, glutamic acid), uncharged polar side chains (for example glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), unpolar side chains (for example alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (for example threonine, valine, isoleucine) and aromatic side chains (for example tyrosine, phenylalanine, tryptophan, histidine). A predicted nonessential amino acid residue in a desaturase or elongase is thus preferably substituted by another amino acid residue from the same family of side chains. As an alternative, the mutations can, in a different embodiment, be introduced randomly over the entire desaturase-encoding sequence or part thereof, for example by means of saturation mutagenesis, and the resulting mutants can be screened for the desaturase activity described herein in order to identify mutants which retain the desaturase or elongase activity. After the mutagenesis of one of the sequences of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 or 31 the encoded protein can be

expressed recombinantly, and the activity of the protein can be determined for example using the assays described herein.

The invention is illustrated further by the examples which follow, but which are not to be construed as limiting. The content of all of the references, patent applications, patents and published patent applications cited in the present patent application is herein incorporated by reference.

Examples section

Example 1: General methods

a) General cloning methods:

Cloning methods such as, for example, restriction cleavages, agarose gel electrophoresis, purification of DNA fragments, transfer of nucleic acids onto nitrocellulose and nylon membranes, linking of DNA fragments, transformation of Escherichia coli and yeast cells, bacterial cultures and sequence analysis of recombinant DNA were carried out as described in Sambrook et al. (1989) (Cold Spring Harbor Laboratory Press: ISBN 0-87969-309-6) or Kaiser, Michaelis and Mitchell (1994) "Methods in Yeast Genetics" (Cold Spring Harbor Laboratory Press: ISBN 0-87969-451-3).

b) Chemicals

Unless otherwise stated in the text, the chemicals used were obtained in analytical-grade quality from Fluka (Neu-Ulm), Merck (Darmstadt), Roth (Karlsruhe), Serva (Heidelberg) and Sigma (Deisenhofen). Solutions were made with purified, pyrogen-free water, hereinbelow referred to as H_2O, from a Milli-Q Water System water purification system (Millipore, Eschborn). Restriction endonucleases, DNA-modifying enzymes and molecular biology kits were obtained from AGS (Heidelberg), Amersham (Braunschweig), Biometra (Göttingen), Boehringer (Mannheim), Genomed (Bad Oeynhausen), New England Biolabs (Schwalbach/Taunus), Novagen (Madison, Wisconsin, USA), Perkin-Elmer (Weiterstadt), Pharmacia (Freiburg), Qiagen (Hilden) and Stratagene (Amsterdam, Netherlands). Unless otherwise specified, they were used in accordance with the manufacturer's instructions.

Example 2: Isolation of total RNA and poly(A)$^+$-RNA from plants

Total RNA is isolated from plants such as linseed and oilseed rape and the like following a method described by Logemann et al. (1987, Anal. Biochem. 163, 21). The total RNA can be obtained from protonemal tissue from moss using the GTC method (Reski et al., 1994, Mol. Gen. Genet., 244:352-359).

Example 3: Transformation of Agrobacterium

The Agrobacterium-mediated transformation of plants can be carried out for example using the Agrobacterium tumefaciens strain GV3101- (pMP90-) (Koncz and Schell, Mol. Gen. Genet. 204 (1986) 383-396) or LBA4404- (Clontech) or C58C1 pGV2260 (Deblaere et al 1984, Nucl. Acids Res. 13, 4777-4788)). The transformation can be carried out by standard transformation techniques (also Deblaere et al. 1984).

Example 4: Plant transformation

The Agrobacterium-mediated transformation of plants can be carried out using standard transformation and regeneration techniques (Gelvin, Stanton B., Schilperoort, Robert A., Plant Molecular Biology Manual, 2nd ed., Dordrecht: Kluwer Academic Publ., 1995, in Sect., Ringbuch Zentrale Signatur: BT11-P ISBN 0-7923-2731-4; Glick, Bernard R., Thompson, John E., Methods in Plant Molecular Biology and Biotechnology, Boca Raton: CRC Press, 1993, 360 S., ISBN 0-8493-5164-2).

Oilseed rape can be transformed by means of cotyledon or hypocotyl transformation (Moloney et al., Plant Cell 8 (1989) 238-242; De Block et al., Plant Physiol. 91 (1989) 694-701). The use of antibiotics for the seletion of agrobacteria and plants depends on the Agrobacterium strain and the binary vector used for the transformation. Normally, oilseed rape is selected using kanamycin as selectable plant marker.

The Agrobacterium-mediated gene transfer into linseed (Linum usitatissimum) can be carried out using for example a technique described by Mlynarova et al. (1994) Plant Cell Report 13:282-285.

The transformation of soya can be carried out using for example a technique described in EP-A-0 0424 047 (Pioneer Hi-Bred International) or in EP-A-0 0397 687, US 5,376,543, US 5,169,770 (University Toledo).

The transformation of plants using particle bombardment, polyethylene glycol mediated DNA uptake or via the silicon carbonate fiber technique is described for example by Freeling and Walbot "The maize handbook" (1993) ISBN 3-540-97826-7, Springer Verlag New York).

Example 5: Plasmids for plant transformation

Binary vectors such as pBinAR (Höfgen and Willmitzer, Plant Science 66 (1990) 221-230) or pGPTV (Becker et al 1992, Plant Mol. Biol. 20:1195-1197) can be used for plant transformation. The binary vectors can be constructed by ligating the cDNA in sense or antisense orientation into T-DNA. 5' of the cDNA, a plant promoter activates cDNA transcription. A polyadenylation sequence is located 3' of the cDNA. The binary vectors can bear different marker genes. In particular, the nptII marker gene, which encodes kanamycin resistance conferred by neomycin phosphotransferase, can be substituted by the herbicide-resistant form of an acetolactate synthase gene (AHAS or ALS). The ALS gene is described in Ott et al., J. Mol. Biol. 1996, 263:359-360. The v-ATPase-c1 promoter can be cloned into plasmid pBin19 or pGPTV and used for the expression of the marker gene by cloning upstream of the ALS coding region. The abovementioned promoter corresponds to a 1153 base-pair fragment from Beta vulgaris (Plant Mol Biol, 1999, 39:463-475). In this context, not only sulfonylureas, but also imidazolinones such as imazethapyr or sulphonylureas may be used as antimetabolites for the selection.

Tissue-specific expression can be achieved using a tissue-specific promoter. For example, seed-specific expression can be achieved by cloning the DC3 or LeB4 or USP promoter or the phaseolin promoter 5' of the cDNA. However, any other seed-specific promoter element such as, for example, the napin or arcelin promoter (Goossens et al. 1999, Plant Phys. 120(4):1095-1103 and Gerhardt et al. 2000, Biochimica et Biophysica Acta 1490(1-2):87-98) may also be used. The CaMV-35S promoter or a v-ATPase C1 promoter can be used for constitutive expression in the intact plants.

In particular, genes encoding desaturases and elongases can be cloned into a binary vector one after the other by constructing a plurality of expression cassettes in order to mimic the metabolic pathway in plants.

Within an expression casette, the protein to be expressed can be targeted into a cellular compartment using a signal peptide, for example for plastids, mitochondria or the endoplasmic reticulum

(Kermode, Crit. Rev. Plant Sci. 15, 4 (1996) 285-423). The signal peptide is cloned 5' in the reading frame with the cDNA to achieve the subcellular localization of the fusion protein.

Examples of multiexpression cassettes are given hereinbelow.

I.) Promoter-terminator cassettes

Expression cassettes consist of least two functional units such as a promoter and a terminator. Further desired gene sequences such as targeting sequences, coding regions of genes or parts thereof and the like can be inserted between promoter and terminator. To construct expression cassettes, promoters and terminators (USP promoter: Baeumlein et al., Mol Gen Genet, 1991, 225 (3):459-67); OCS terminator: Gielen et al. EMBO J. 3 (1984) 835ff.) are isolated with the aid of the polymerase chain reaction and tailor-made with flanking sequences of choice on the basis of synthetic oligonucleotides.

Examples of oligonucleotides which can be used are the following:

USP1 upstream: CCGGAATTCGGCGCGCCGAGCTCCTCGAGCAAATTTACACATTGCCA

USP2 upstream: CCGGAATTCGGCGCGCCGAGCTCCTCGAGCAAATTTACACATTGCCA

USP3 upstream: CCGGAATTCGGCGCGCCGAGCTCCTCGAGCAAATTTACACATTGCCA

USP1 downstream: AAAACTGCAGGCGGCCGCCCACCGCGGTGGGCTGGCTATGAAGAAATT

USP2 downstream: CGCGGATCCGCTGGCTATGAAGAAATT

USP3 downstream: TCCCCCGGGATCGATGCCGGCAGATCTGCTGGCTATGAAGAAATT

OCS1 upstream: AAAACTGCAGTCTAGAAGGCCTCCTGCTTTAATGAGATAT

OCS2 upstream:
CGCGGATCCGATATCGGGCCCGCTAGCGTTAACCCTGCTTTAATGAGATAT

OCS3 upstream: TCCCCCGGGCCATGGCCTGCTTTAATGAGATAT

OCS1 downstream:
CCCAAGCTTGGCGCGCCGAGCTCGAATTCGTCGACGGACAATCAGTAAATTGA

OCS2 downstream:
CCCAAGCTTGGCGCGCCGAGCTCGAATTCGTCGACGGACAATCAGTAAATTGA

OCS3 downstream: CCCAAGCTTGGCGCGCCGAGCTCGTCGACGGACAATCAGTAAATTGA

The methods are known to the specialist worker and are generally known from the literature.

In a first step, a promoter and a terminator are amplified via PCR. Then, the terminator is cloned into a recipient plasmid and, in a second step, the promoter is inserted upstream of the terminator. This gives an expression cassette on a plasmid vehicle. The plasmids pUT1, 2 and 3 are generated on the basis of the plasmid pUC19.

The constructs are defined in accordance with the invention in SEQ ID NO: 33, 34 and 42. They comprise the USP promoter and the OCS terminator. Based on these plasmids, the construct pUT12 is generated by cutting pUT1 with SalI/ScaI and cutting pUT2 with XhoI/ScaI. The fragments in the expression cassettes are ligated and transformed into E. coli XLI blue MRF. After picking out ampicillin-resistant colonies, DNA is prepared, and those clones which comprise two expression cassettes are identified by restriction analysis. The XhoI/SalI ligation of compatible ends has eliminated the two cleavage sites XhoI and SalI between the expression cassettes. This gives rise to plasmid pUT12, which is defined in SEQ ID NO: 36. pUT12 is subsequently cut again with SalI/ScaI and pUT3 with XhoI/ScaI. The fragments comprising the expression cassettes are ligated and transformed into E. coli XLI blue MRF. After singling out ampicillin-resistant colonies, DNA is prepared, and those clones which comprise three expression cassettes are identified by restriction analysis. In this manner, a set of multiexpression cassettes is created which can be exploited for inserting the desired DNA and is described in Table 1 and can additionally incorporate further expression cassettes.

They comprise the following elements:

Table 1

pUC19 derivate	Cleavage sites before the USP promoter	Multiple cloning cleavage sites	Cleavage sites behind the OCS terminator
pUT1	EcoRI/AscI/ SacI/XhoI	BstXI/NotI/ PstI/XbaI/StuI	SalI/EcoRI/ SacI/AscI/ HindIII
pUT2	EcoRI/AscI/ SacI/XhoI	BamHI/EcoRV/ ApaI/NheI/ HpaI	SalI/EcoRI/ SacI/AscI/ HindIII
pUT3	EcoRI/AscI/ SacI/XhoI	BglII/NaeI/ ClaI/SmaI/NcoI	SalI/SacI/ AscI/HindIII
pUT12 Double expression cassette	EcoRI/AscI/ SacI/XhoI	BstXI/NotI/ PstI/XbaI/StuI and BamHI/EcoRV/ ApaI/NheI/ HpaI	SalI/EcoRI/ SacI/AscI/ HindIII
pUT123 Triple expression cassette	EcoRI/AscI/ SacI/XhoI	1.BstXI/NotI/ PstI/XbaI/StuI and 2.BamHI/EcoRV/ ApaI/NheI/ HpaI and 3.BglII/NaeI/ ClaI/SmaI/NcoI	SalI/SacI/AscI/IIindIII

Furthermore, further multiexpression cassettes can be generated and employed for seed-specific gene expression, as described and as specified in greater detail in Table 2, with the aid of the

i) USP promoter or with the aid of the
ii) 700 base pair 3' fragment of the LeB4 promoter or with the aid of the
iii) DC3 promoter.

The DC3 promoter is described in Thomas, Plant Cell 1996, 263:359-368 and consists merely of the region -117 to +26, which is why it therefore constitutes one of the smallest known seed-specific promoters. The expression cassettes can comprise several copies of the same promoter or else be constructed via three different promoters.

The vectors used for the transformation of plants and the sequences of the inserted genes/proteins can be found in sequence listing SEQ ID NO: 43 to 49.

Advantageously used polylinker or polylinker-terminator-polylinkers can be found in the sequences SEQ ID NO: 50 to 52.

Table 2: Multiple expression cassettes

Plasmid name of the pUC19 derivative	Cleavage sites before the respective promoter	Multiple cloning cleavage sites	Cleavage sites behind the OCS terminator
pUT1 (pUC19 with USP-OCS1)	EcoRI/AscI/SacI/XhoI	(1) BstXI/NotI/PstI/ XbaI/StuI	SalI/EcoRI/SacI/AscI/ HindIII
pDCT (pUC19 with DC3-OCS)	EcoRI/AscI/SacI/XhoI	(2) BamHI/EcoRV/ ApaI/NheI/ HpaI	SalI/EcoRI/SacI/AscI/ HindIII
pLeBT (pUC19-with LeB4(700)-OCS)	EcoRI/AscI/SacI/XhoI	(3) BglII/NaeI/ ClaI/SmaI/NcoI	SalI/SacI/AscI/HindIII
pUD12 (pUC 19 with USP-OCS1 and with DC3-OCS)	EcoRI/AscI/SacI/XhoI	(1) BstXI/NotI/ PstI/XbaI/StuI and (2) BamHI/EcoRV/ ApaI/NheI/ HpaI	SalI/EcoRI/SacI/AscI/ HindIII
pUDL123 Triple expression cassette (pUC19 with USP/DC3 and LeB4-700)	EcoRI/AscI/SacI/XhoI	(1) BstXI/NotI/ PstI/XbaI/StuI and (2) BamHI/ (EcoRV*)/ApaI/ NheI/HpaI and (3) BglII/NaeI/ ClaI/SmaI/NcoI	SalI/SacI/AscI/HindIII

* EcoRV cleavage site in the 700 base-pair fragment of the LeB4 promoter (LeB4-700)

Further promoters for multi-gene constructs can be generated analogously, in particular using the

a) 2.7 kb fragment of the LeB4 promoter or with the aid of the
b) phaseolin promoter or with the aid of the
c) constitutive v-ATPase c1 promoter.

It may be particularly desirable to use further especially suitable promoters for constructing seed-specific multi-expression cassettes such as, for example, the napin promoter or the arcelin-5 promoter.

II) Generation of expression constructs which comprise promoter, terminator and desired gene sequence for the expression of PUFA genes in plant expression cassettes.

In pUT123, the Δ6-elongase Pp_PSE1 is first inserted into the first cassette via BstXI and XbaI. Then, the moss Δ-6-desaturase (Pp_des6) is inserted into the second cassette via BamHI/NaeI,

and, finally, the Phaeodactylum Δ5-desaturase (Pt_des5) is inserted into the third cassette via BglII/NcoI. The triple construct is named pARA1. Taking into consideration sequence-specific restriction cleavage sites, further expression cassettes are shown in Table 3, which are named pARA2, pARA3 and pARA4, can be generated.

Table 3: Combinations of desaturases and elongases

Gene plasmid	Δ6-Desaturase	Δ5-Desaturase	Δ6-Elongase
pARA1	Pp_des6	Pt_des5	Pp_PSE1
pARA2	Pt_des6	Pt_des5	Pp_PSE1
pARA3	Pt_des6	Ce_des5	Pp_PSE1
pARA4	Ce_des6	Ce_des5	Ce_PSE1

Pp = Physcomitrella patens, Pt = Phaeodactylum tricornutum
Pp_PSE1 corresponds to the sequence of SEQ ID NO: 9.
PSE = PUFA-specific Δ6-elongase
Ce_des5 = Δ5-desaturase from Caenorhabditis elegans (Genbank Acc. No. AF078796)
Ce_des6 = Δ6-desaturase from Caenorhabditis elegans elegans (Genbank Acc. No. AF031477, bases 11-1342)
Ce_PSE1 = Δ6-elongase from Caenorhabditis elegans (Genbank Acc. No. AF244356, bases 1-867)

Further desaturases or elongase sequences can also be inserted into the expression cassettes in the described manner, such as, for example, Genbank Acc. Nr. AF231981, NM_013402, AF206662, AF268031, AF226273, AF110510 or AF110509.

iii) Transfer of expression cassettes into vectors for the transformation of Agrobacterium tumefaciens and for the transformation of plants

The constructs generated thus are inserted into the binary vector pGPTV by means of AscI. For this purpose, the multiple cloning sequence is extended by an AscI cleavage site. For this purpose, the polylinker is synthesized *de novo* as two double-stranded oligonucleotides, thereby introducing an additional AscI DNA sequence. The oligonucleotide is inserted into the vector pGPTV by means of EcoRI and HindIII. The cloning techniques required are known to the skilled worker and can simply be found in the literature as described in Example 1.

Example 6: Studying the expression of a recombinant gene product in a transformed organism

The activity of a recombinant gene product in the transformed host organism can be measured at the transcriptional and/or the translational level.

A suitable method for determining the extent to which the gene is transcribed (which indicates the amount of RNA which is available for the translation of the gene product) is to carry out a Northern blot as detailed hereinbelow (as reference, see Ausubel et al. (1988) Current Protocols in Molecular Biology, Wiley: New York, or the abovementioned Examples Section), where a primer which is designed in such a way that it binds to the gene of interest is labeled with a detectable label (usually a radioactive label or a chemiluminescent label) so that, when the total RNA of a culture of the organism is extracted, separated on a gel, transferred onto a stable matrix and incubated with this probe, the binding and extent of the binding of the probe indicates the existence and also the amount of the mRNA for this gene. This information indicates the degree to which the transformed gene has been transcribed. Cellular total RNA can be prepared from cells, tissues or organs using a plurality of methods, all of which are known in the art, such as, for example, the method of Bormann, E.R., et al. (1992) Mol. Microbiol. 6:317-326.

Northern hybridization:

To carry out the RNA hybridization, 20 µg of total RNA or 1 µg of poly(A)$^+$ RNA were separated by gel electrophoresis in agarose gels with a strength of 1.25% using formaldehyde, as described in Amasino (1986, Anal. Biochem. 152, 304), capillary-blotted onto positively charged nylon membranes Hybond N+, Amersham, Braunschweig) using 10 x SSC, immobilized using UV-light and prehybridized for 3 hours at 68°C using hybridization buffer (10% dextran sulfate w/v, 1 M NaCl, 1% SDS, 100 mg herring sperm DNA). The DNA probe was labeled with the Highprime DNA labeling kit (Roche, Mannheim, Germany) during the prehybridization step, using alpha-^{32}P-dCTP (Amersham, Braunschweig, Germany). After the labeled DNA probe had been added, the hybridization was carried out overnight at 68°C in the same buffer. The wash steps were carried out twice for 15 minutes using 2 x SSC and twice for 30 minutes using 1 x SSC, 1% SDS, at 68°C. The sealed filters were exposed at -70°C for a period of 1 to 14 days.

Standard techniques, such as a Western blot, can be employed for studying the presence or the relative amount of protein translated by this mRNA (see, for example, Ausubel et al. (1988) Current Protocols in Molecular Biology, Wiley: New York). In this method, the cellular total proteins are extracted, separated by gel electrophoresis, transferred to a matrix such as nitrocellulose and incubated with a probe such as an antibody which binds specifically to the desired protein. This probe is usually provided with a chemiluminescent or colorimetric label which can be detected readily. The presence and the amount of the label observed indicates the presence and the amount of the desired mutated protein present in the cell.

Example 7: Analysis of the effect of the recombinant proteins on the production of the desired product

The effect of the genetic modification in plants, fungi, algae, ciliates or on the production of a desired compound (such as a fatty acid) can be determined by growing the modified microorganisms or the modified plant under suitable conditions (such as those described above) and analyzing the medium and/or the cellular components for the increased production of the desired product (i.e. of lipids or a fatty acid). These analytical techniques are known to the skilled worker and comprise spectroscopy, thin-layer chromatography, various tyes of staining methods, enzymatic and microbiological methods, and analytical chromatography such as high-performance liquid chromatography (see, for example, Ullman, Encyclopedia of Industrial Chemistry, vol. A2, pp. 89-90 and pp. 443-613, VCH: Weinheim (1985); Fallon, A., et al., (1987) "Applications of HPLC in Biochemistry" in: Laboratory Techniques in Biochemistry and Molecular Biology, vol. 17; Rehm et al. (1993) Biotechnology, vol. 3, chapter III: "Product recovery and purification", pp. 469-714, VCH: Weinheim; Belter, P.A., et al. (1988) Bioseparations: downstream processing for Biotechnology, John Wiley and Sons; Kennedy, J.F. and Cabral, J.M.S. (1992) Recovery processes for biological Materials, John Wiley and Sons; Shaeiwitz, J.A., and Henry, J.D. (1988) Biochemical Separations, in: Ullmann's Encyclopedia of Industrial Chemistry, vol. B3; chapter 11, pp. 1-27, VCH: Weinheim; and Dechow, F.J. (1989) Separation and purification techniques in biotechnology, Noyes Publications).

In addition to the abovementioned methods, plant lipids are extracted from plant material as described by Cahoon et al. (1999) Proc. Natl. Acad. Sci. USA 96 (22):12935-12940, and Browse et al. (1986) Analytic Biochemistry 152:141-145. The qualitative

and quantitative lipid and fatty acid analysis is described by Christie, William W., Advances in Lipid Methodology, Ayr/Scotland: Oily Press (Oily Press Lipid Library; 2); Christie, William W., Gas Chromatography and Lipids. A Practical Guide - Ayr, Scotland: Oily Press, 1989, Repr. 1992, IX, 307 S. (Oily Press Lipid Library; 1); "Progress in Lipid Research, Oxford: Pergamon Press, 1 (1952) - 16 (1977) u.d.T.: Progress in the Chemistry of Fats and Other Lipids CODEN.

To determine the overall efficiency with which the compound is produced, it is also possible, in addition to measuring the fermentation end product, to analyze other components of the metabolic pathways which are used for producing the desired compounds, such as intermediates and secondary products. The analytical methods comprise measurements of the nutrient quantities in the medium (for example sugars, hydrocarbons, nitrogen sources, phosphate and other ions), measurements of the biomass composition and the growth, analysis of the production of usual metabolites via biosynthetic pathways, and measurements of gases which are generated during the fermentation process. Standard methods for these measurements are described in Applied Microbial Physiology; A Practical Approach, P.M. Rhodes and P.F. Stanbury, ed., IRL Press, pp. 103-129; 131-163 and 165-192 (ISBN: 0199635773) and references cited therein.

One example is the analysis of fatty acids (abbreviations: FAMEs, fatty acid methyl esters; GC-MS, gas liquid chromatography/mass spectrometry; TAG, triacylglycerol; TLC, thin-layer chromatography).

Unequivocal proof for the presence of fatty acid products can be obtained by the analysis of recombinant organisms following standard analytical procedures: GC, GC-MS or TLC as variously described by Christie and references therein (1997, in: Advances on Lipid Methodology, Fourth ed.: Christie, Oily Press, Dundee, 119-169; 1998, gas-chromatography/mass-spectrometry methods, Lipids 33:343-353).

Material to be analyzed can be disintegrated via sonification, glass milling, liquid nitrogen and grinding or via other applicable methods. The material has to be centrifuged after disintegration. The sediment is resuspended in Aqua dest, heated for 10 min at 100°C, cooled on ice and centrifuged again, followed by extraction in 0.5 M sulfuric acid in methanol containing 2% dimethoxypropane for 1 h at 90°C, leading to hydrolyzed oil and liquid compounds, resulting in transmethylated lipids. These fatty acid methyl esters are extracted in petrolether and finally

subjected to GC analysis using a capillary column (Chrompack, WCOT Fused Silica, CP-Wax-52 CB, 25 µm, 0.32 mm) at a temperature gradient beteen 170°C and 240°C for 20 min and 5 min at 240°C. The identity of resulting fatty acid methylesters has to be defined by the use of standards available from commercial sources (i.e. Sigma).

In the case of fatty acids where standards are not available molecule identity has to be shown via derivatization and subsequent GC MS analysis. For example the localization of triple bond fatty acids has to be shown via GC-MS after derivatization via 4,4-dimethoxyoxazoline derivatives (Christie, 1998, see above).

Expression constructs in heterologous microbial systems

Strains, Growth Conditions and Plasmids

Escherichia coli strain XL1 Blue MRF' kan (Stratagene) was used for sub-cloning the new elongase pPDesaturase1 from Physcomitrella patens. For functional expression of this gene we used the Saccharomyces cerevisiae strain INVSc 1 (Invitrogen Co.). E. coli was grown in Luria-Bertini broth (LB, Duchefa, Haarlem, The Netherlands) at 37°C. When necessary, ampicillin (100 mg/liter) was added and 1.5% (w/v) agar was included for solid LB media. S. cerevisiae was grown at 30°C either in YPG-medium or in complete minimal dropout uracil medium (CMdum; see in: Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., Struhl, K., Albright, L.B., Coen, D.M., and Varki, A. (1995) Current Protocols in Molecular Biology, John Wiley & Sons, New York) containing either 2% (w/v) raffinose or glucose. For solid media 2% (w/v) Bacto™ agar (Difco) was included. Plasmids used for cloning and expression were pUC18 (Pharmacia) and pYES2 (Invitrogen Co.).

Example 8: Cloning and expression of PUFA-specific desaturases and elongases

For expression in plants, cDNA clones from SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31 were modified in such a way that only the coding region was amplified by means of polymerase chain reaction using two oligonucleotides. Care was taken that a consensus sequence before the start codon was maintained for efficient translation. To this end, either the base sequence ATA or AAA was chosen and introduced into the sequence before the ATG (Kozak, M. (1986) Point mutations define a sequence flanking the AUG initiator codon that modulates

translation by eukaryotic ribosomes, Cell 44, 283-292). In addition, a restriction cleavage site was introduced before this consensus triplet, which restriction cleavage site must be compatible with the cleavage site of the target vector into which the fragment is to be cloned and with the aid of which the expression of genes in microorganisms or plants is to take place.

The PCR reaction was performed with plasmid DNA as template in a Thermocycler (Biometra) using the Pfu-DNA (Stratagene) polymerase and the following temperature programme: 3 minutes at 96°C, followed by 30 cycles with 30 seconds at 96°C, 30 seconds at 55°C and 2 minutes at 72°C, 1 cycle with 10 minutes at 72°C and stop at 4°C. The annealing temperature was varied, depending on the oligonucleotides chosen. A synthesis time of approximately one minute can be assumed per kilobase pairs DNA. Further parameters which have an effect on the PCR such as, for example, Mg ions, salt, DNA polymerase and the like are known to the specialist worker and can be varied as required.

The correct size of the amplified DNA fragment was verified by agarose-TBE gel electrophoresis. The amplified DNA was extracted from the gel using the QIAquick Gel Extraction Kit (QIAGEN) and ligated into the SmaI restriction site of the dephosphorylated vector pUC18 using the Sure Clone Ligation Kit (Pharmacia), giving rise to the pUC derivatives. After the transformation of E. coli XL1 Blue MRF' kan, a DNA miniprep (Riggs, M.G., & McLachlan, A. (1986) A simplified screening procedure for large numbers of plasmid mini-preparation. BioTechniques 4, 310-313) was carried out on ampicillin-resistant transformants, and positive clones were identified by means of BamHI restriction analysis. The sequence of the cloned PCR product was verified by resequencing using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Weiterstadt).

Fatty acid analysis

The total fatty acids were extracted from plant seeds and analyzed by gas chromatography.

The seeds were taken up in 1% sodium methoxide in methanol and incubated for 20 minutes at RT. Thereafter, the mixture is washed with NaCl solution, and the FAMEs are taken up in 0.3 ml heptane. The samples were separated on a ZEBRON-ZB-Wax capillary column (30 m, 0.32 mm, 0.25 µm; Phenomenex) in a Hewlett Packard-6850 gas chromatograph with flame ionization detector. The oven temperature was programmed from 70°C (1 minute hold) to 200°C at a rate of 20°C/minute, then to 250°C (5 min hold) at a rate of 5°C/min and finally to 260°C at a rate of 5°C/min. Nitrogen was

used as carrier gas (4.5 ml/min at 70°C). The fatty acids were identified by comparing the retention times with those of FAME standards (SIGMA).

Expression analysis

Result of the expression of a Phaeodactylum tricornutum Δ6-acyl-lipid desaturase, a Phaeodactylum tricornutum Δ5-acyl-lipid desaturase and the delta-6-specific elongase in tobacco seeds:

Figure 2: Fatty acid profile of transgenic tobacco seeds. The plants were transformed with a triple expression cassette which expresses, under the control of the USP promoter, the delta-6-, the delta-5- and the Physcomitrella patens PpPSE1 (pARA2). 100 transgenic tobacco and linseed plants are generated, of which approximately 20% synthesize arachidonic acid in the seed.

Figure 3: Tobacco wild-type control.

Example 9: Purification of the desired product from transformed organisms

The desired product can be obtained from plant material or fungi, algae, ciliates, animal cells or from the supernatant of the above-described cultures by various methods known in the art. If the desired product is not excreted from the cells, the cells can be harvested from the culture by slow centrifugation, and the cells can be lyzed by standard techniques such as mechanical force or sonication. Plant organs can be separated mechanically from other tissue or other organs. After homogenization, the cell debris is removed by centrifugation, and the supernatant fraction, which comprises the soluble proteins, is stored for the further purification of the desired compound. If the product is excreted from desired cells, the cells are removed from the culture by slow centrifugation, and the supernatant fraction is stored for further purification.

The supernatant fraction of each purification method is subjected to chromatography with a suitable resin, the desired molecule either being retained on the chromatography resin, while many contaminations in the sample are not, or else the contaminations are retained on the resin, while the sample is not. If necessary, these chromatography steps can be repeated, using identical or different chromatography resins. The skilled worker is familiar with the selection of suitable chromatography resins and their most effective application for a particular molecule to be

purified. The purified product can be concentrated by filtration or ultrafiltration and stored at a temperature which provides maximum stability of the product.

A broad spectrum of purification methods is known in the art, and the above purification method is not intended to be limiting. These purification methods are described, for example, in Bailey, J.E., & Ollis, D.F., Biochemical Engineering Fundamentals, McGraw-Hill: New York (1986).

The identity and purity of the compounds which have been isolated can be determined by standard techniques of the art. These include high-performance liquid chromatography (HPLC), spectroscopic methods, staining methods, thin-layer chromatography, in particular thin-layer chromatography and flame ionization detection (IATROSCAN, Iatron, Tokio, Japan), NIRS, enzyme assay or microbiological methods. For an overview of these analytical methods, see: Patek et al. (1994) Appl. Environ. Microbiol. 60:133-140; Malakhova et al. (1996) Biotekhnologiya 11:27-32; and Schmidt et al. (1998) Bioprocess Engineer. 19:67-70. Ulmann's Encyclopedia of Industrial Chemistry (1996) vol. A27, VCH: Weinheim, pp. 89-90, pp. 521-540, pp. 540-547, pp. 559-566, 575-581 and pp. 581-587; Michal, G (1999) Biochemical Pathways: An Atlas of Biochemistry and Molecular Biology, John Wiley and Sons; Fallon, A., et al. (1987) Applications of HPLC in Biochemistry in: Laboratory Techniques in Biochemistry and Molecular Biology, vol. 17.

Equivalents

The skilled worker will or can recognize many equivalents of the specific embodiments according to the invention described herein by simply using routine experiments. These equivalents are intended to fall within the patent claims.

Process for the production of polyunsaturated fatty acids in plants

Abstract

The present invention relates to a method for the production of fatty acid esters which comprise unsaturated fatty acids with at least three double bonds, and to free unsaturated fatty acids with a content of at least 1% by weight based on the total fatty acids present in the plants, by expressing at least one nucleic acid sequence which encodes a polypeptide with Δ6-desaturase activity and at least one nucleic acid sequence which encodes a polypeptide with Δ6-elongase activity. Advantageously, these nucleic acid sequences can, if appropriate, be expressed in the transgenic plant together with a third nucleic acid sequence which encodes a polypeptide with Δ5-desaturase activity.

The invention furthermore relates to the use of defined nucleic acid sequences which encode polypeptides with a Δ6-desaturase activity, Δ6-elongase activity or Δ5-desaturase activity selected from a group of nucleic acid sequences, and/or to the use of nucleic acid constructs comprising the abovementioned nucleic acid sequences.

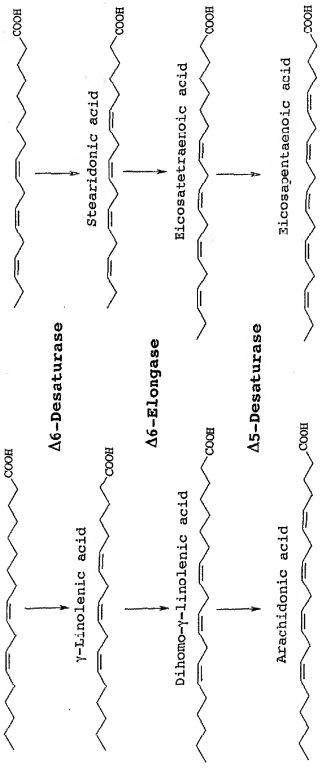


Figure 1: Biosynthesis chain

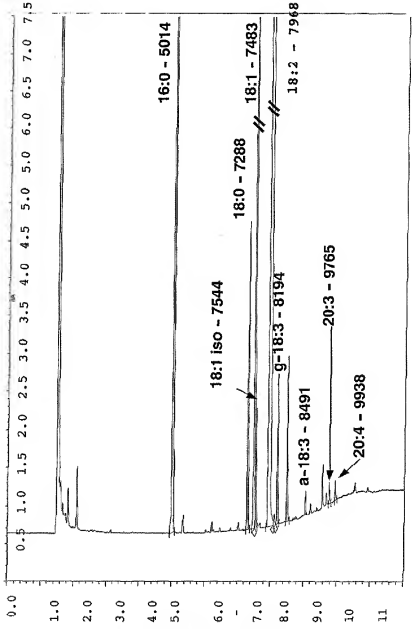


Figure 2: Fatty acid profile of transgenic tobacco seeds

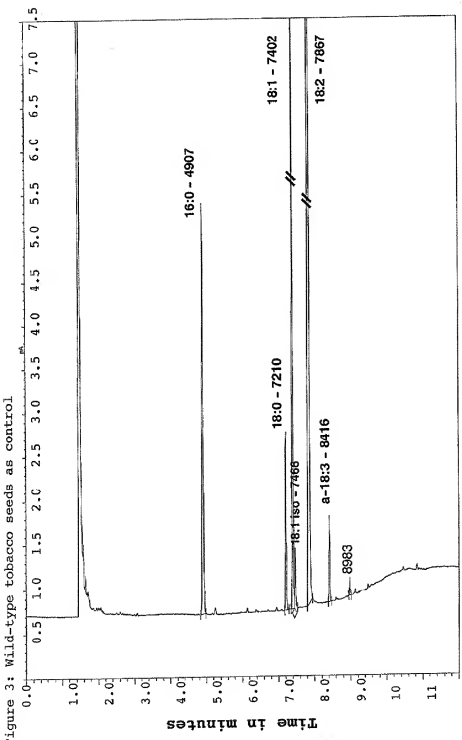


Figure 3: Wild-type tobacco seeds as control

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ctc caa gct ttg gtg ctt gtg cac aac ctg ttc tgt ttt gcg ctc agt 345

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Leu Gln Ala Leu Val Leu Val His Asn Leu Phe Cys Phe Ala Leu Ser
                85                  90                  95

ctg tat atg tgc gtg ggc atc gct tat cag gct att acc tgg cgg tac   393
Leu Tyr Met Cys Val Gly Ile Ala Tyr Gln Ala Ile Thr Trp Arg Tyr
            100                 105                 110

tct ctc tgg ggc aat gca tac aat cct aaa cat aaa gag atg gcg att   441
Ser Leu Trp Gly Asn Ala Tyr Asn Pro Lys His Lys Glu Met Ala Ile
        115                 120                 125

ctg gta tac ttg ttc tac atg tct aag tac gtg gaa ttc atg gat acc   489
Leu Val Tyr Leu Phe Tyr Met Ser Lys Tyr Val Glu Phe Met Asp Thr
    130                 135                 140

gtt atc atg ata ctg aag cgc agc acc agg caa ata agc ttc ctc cac   537
Val Ile Met Ile Leu Lys Arg Ser Thr Arg Gln Ile Ser Phe Leu His
145                 150                 155                 160

gtt tat cat cat tct tca att tcc ctc att tgg tgg gct att gct cat   585
Val Tyr His His Ser Ser Ile Ser Leu Ile Trp Trp Ala Ile Ala His
                165                 170                 175

cac gct cct ggc ggt gaa gca tat tgg tct gcg gct ctg aac tca gga   633
His Ala Pro Gly Gly Glu Ala Tyr Trp Ser Ala Ala Leu Asn Ser Gly
            180                 185                 190

gtg cat gtt ctc atg tat gcg tat tac ttc ttg gct gcc tgc ctt cga   681
Val His Val Leu Met Tyr Ala Tyr Tyr Phe Leu Ala Ala Cys Leu Arg
        195                 200                 205

agt agc cca aag tta aaa aat aag tac ctt ttt tgg ggc agg tac ttg   729
Ser Ser Pro Lys Leu Lys Asn Lys Tyr Leu Phe Trp Gly Arg Tyr Leu
    210                 215                 220

aca caa ttc caa atg ttc cag ttt atg ctg aac tta gtg cag gct tac   777
Thr Gln Phe Gln Met Phe Gln Phe Met Leu Asn Leu Val Gln Ala Tyr
225                 230                 235                 240

tac gac atg aaa acg aat gcg cca tat cca caa tgg ctg atc aag att   825
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Tyr Asp Met Lys Thr Asn Ala Pro Tyr Pro Gln Trp Leu Ile Lys Ile
245 250 255

ttg ttc tac tac atg atc tcg ttg ctg ttt ctt ttc ggc aat ttt tac 873
Leu Phe Tyr Tyr Met Ile Ser Leu Leu Phe Leu Phe Gly Asn Phe Tyr
260 265 270

gta caa aaa tac atc aaa ccc tct gac gga aag caa aag gga gct aaa 921
Val Gln Lys Tyr Ile Lys Pro Ser Asp Gly Lys Gln Lys Gly Ala Lys
275 280 285

act gag tga gctgtatcaa gccatagaaa ctctattatg ttagaacctg 970
Thr Glu
290

aagttggtgc tttcttatct ccacttatct tttaagcagc atcagttttg aaatgatgtg 1030

tgggcgtggt ctgcaagtag tcatcaatat aatcggcctg agcacttcag atggattgtt 1090

agaacatgag taaaagcggt tattacggtg tttattttgt accaaatcac cgcacgggtg 1150

aattgaaata tttcagattt gatcaatttc atctgaaaaa aa 1192

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20 25 30

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35 40 45

Val Leu Gly Val Ser Val Tyr Leu Thr Ile Val Ile Gly Gly Leu Leu

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     50                  55                  60

Trp Ile Lys Ala Arg Asp Leu Lys Pro Arg Ala Ser Glu Pro Phe Leu
 65                  70                  75                  80

Leu Gln Ala Leu Val Leu Val His Asn Leu Phe Cys Phe Ala Leu Ser
                85                  90                  95

Leu Tyr Met Cys Val Gly Ile Ala Tyr Gln Ala Ile Thr Trp Arg Tyr
            100                 105                 110

Ser Leu Trp Gly Asn Ala Tyr Asn Pro Lys His Lys Glu Met Ala Ile
        115                 120                 125

Leu Val Tyr Leu Phe Tyr Met Ser Lys Tyr Val Glu Phe Met Asp Thr
    130                 135                 140

Val Ile Met Ile Leu Lys Arg Ser Thr Arg Gln Ile Ser Phe Leu His
145                 150                 155                 160

Val Tyr His His Ser Ser Ile Ser Leu Ile Trp Trp Ala Ile Ala His
                165                 170                 175

His Ala Pro Gly Gly Glu Ala Tyr Trp Ser Ala Ala Leu Asn Ser Gly
            180                 185                 190

Val His Val Leu Met Tyr Ala Tyr Tyr Phe Leu Ala Ala Cys Leu Arg
        195                 200                 205

Ser Ser Pro Lys Leu Lys Asn Lys Tyr Leu Phe Trp Gly Arg Tyr Leu
    210                 215                 220

Thr Gln Phe Gln Met Phe Gln Phe Met Leu Asn Leu Val Gln Ala Tyr
225                 230                 235                 240

Tyr Asp Met Lys Thr Asn Ala Pro Tyr Pro Gln Trp Leu Ile Lys Ile
                245                 250                 255

Leu Phe Tyr Tyr Met Ile Ser Leu Leu Phe Leu Phe Gly Asn Phe Tyr
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Thr Glu
290

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Met Met Glu Pro
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Leu Asp Arg Tyr Arg Ala Leu Ala Glu Leu Ala Ala Arg Tyr Ala Ser
5 10 15 20

tcg gcg gcc ttc aag tgg caa gtc acg tac gac gcc aag gac agc ttc 150
Ser Ala Ala Phe Lys Trp Gln Val Thr Tyr Asp Ala Lys Asp Ser Phe
25 30 35

gtc ggg ccc ctg cga atc cgg gag ccg ctc ggg ctc ctg gtg ggc tcc 198
Val Gly Pro Leu Gly Ile Arg Glu Pro Leu Gly Leu Leu Val Gly Ser
40 45 50

gtg gtc ctc tac ctg agc ctg ctg gcc gtg gtc tac gcg ctg cgg aac 246
Val Val Leu Tyr Leu Ser Leu Leu Ala Val Val Tyr Ala Leu Arg Asn
55 60 65

tac ctt ggc ggc ctc atg gcg ctc cgc agc gtg cat aac ctc ggg ctc 294

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Tyr Leu Gly Gly Leu Met Ala Leu Arg Ser Val His Asn Leu Gly Leu
     70                  75                  80

tgc ctc ttc tcg ggc gcc gtg tgg atc tac acg agc tac ctc atg atc   342
Cys Leu Phe Ser Gly Ala Val Trp Ile Tyr Thr Ser Tyr Leu Met Ile
 85                  90                  95                 100

cag gat ggg cac ttt cgc agc ctc gag gcg gca acg tgc gag ccg ctc   390
Gln Asp Gly His Phe Arg Ser Leu Glu Ala Ala Thr Cys Glu Pro Leu
                105                 110                 115

aag cat ccg cac ttc cag ctc atc agc ttg ctc ttt gcg ctg tcc aag   438
Lys His Pro His Phe Gln Leu Ile Ser Leu Leu Phe Ala Leu Ser Lys
            120                 125                 130

atc tgg gag tgg ttc gac acg gtg ctc ctc atc gtc aag ggc aac aag   486
Ile Trp Glu Trp Phe Asp Thr Val Leu Leu Ile Val Lys Gly Asn Lys
        135                 140                 145

ctc cgc ttc ctg cac gtc ttg cac cac gcc acg acc ttt tgg ctc tac   534
Leu Arg Phe Leu His Val Leu His His Ala Thr Thr Phe Trp Leu Tyr
    150                 155                 160

gcc atc gac cac atc ttt ctc tcg tcc atc aag tac ggc gtc gcg gtc   582
Ala Ile Asp His Ile Phe Leu Ser Ser Ile Lys Tyr Gly Val Ala Val
165                 170                 175                 180

aat gct ttc atc cac acc gtc atg tac gcg cac tac ttc cgc cca ttc   630
Asn Ala Phe Ile His Thr Val Met Tyr Ala His Tyr Phe Arg Pro Phe
                185                 190                 195

ccg aag ggc ttg cgc ccg ctt att acg cag ttg cag atc gtc cag ttc   678
Pro Lys Gly Leu Arg Pro Leu Ile Thr Gln Leu Gln Ile Val Gln Phe
            200                 205                 210

att ttc agc atc ggc atc cat acc gcc att tac tgg cac tac gac tgc   726
Ile Phe Ser Ile Gly Ile His Thr Ala Ile Tyr Trp His Tyr Asp Cys
        215                 220                 225

gag ccg ctc gtg cat acc cac ttt tgg gaa tac gtc acg ccc tac ctt   774
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Glu Pro Leu Val His Thr His Phe Trp Glu Tyr Val Thr Pro Tyr Leu
230 235 240

ttc gtc gtg ccc ttc ctc atc ctc ttt ttc aat ttt tac ctg cag cag 822
Phe Val Val Pro Phe Leu Ile Leu Phe Phe Asn Phe Tyr Leu Gln Gln
245 250 255 260

tac gtc ctc gcg ccc gca aaa acc aag aag gca tag ccacgtaaca 868
Tyr Val Leu Ala Pro Ala Lys Thr Lys Lys Ala
265 270

gtagaccagc agcgccgagg acgcgtgccg cgttatcgcg aagcacgaaa taaagaagat 928

catttgattc aacgaggcta cttgcggcca cgagaaaaaa aaaaaaaaaa aaaaaaaaaa 988

aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1048

ctcgag 1054

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Lys Asp Ser Phe Val Gly Pro Leu Gly Ile Arg Glu Pro Leu Gly Leu
35 40 45

Leu Val Gly Ser Val Val Leu Tyr Leu Ser Leu Leu Ala Val Val Tyr
50 55 60

Ala Leu Arg Asn Tyr Leu Gly Gly Leu Met Ala Leu Arg Ser Val His
65 70 75 80

Asn Leu Gly Leu Cys Leu Phe Ser Gly Ala Val Trp Ile Tyr Thr Ser
85 90 95

Tyr Leu Met Ile Gln Asp Gly His Phe Arg Ser Leu Glu Ala Ala Thr
100 105 110

Cys Glu Pro Leu Lys His Pro His Phe Gln Leu Ile Ser Leu Leu Phe
115 120 125

Ala Leu Ser Lys Ile Trp Glu Trp Phe Asp Thr Val Leu Leu Ile Val
130 135 140

Lys Gly Asn Lys Leu Arg Phe Leu His Val Leu His His Ala Thr Thr
145 150 155 160

Phe Trp Leu Tyr Ala Ile Asp His Ile Phe Leu Ser Ser Ile Lys Tyr
165 170 175

Gly Val Ala Val Asn Ala Phe Ile His Thr Val Met Tyr Ala His Tyr
180 185 190

Phe Arg Pro Phe Pro Lys Gly Leu Arg Pro Leu Ile Thr Glu Leu Gln
195 200 205

Ile Val Gln Phe Ile Phe Ser Ile Gly Ile His Thr Ala Ile Tyr Trp
210 215 220

His Tyr Asp Cys Glu Pro Leu Val His Thr His Phe Trp Glu Tyr Val
225 230 235 240

Thr Pro Tyr Leu Phe Val Val Pro Phe Leu Ile Leu Phe Phe Asn Phe
245 250 255

Tyr Leu Gln Gln Tyr Val Leu Ala Pro Ala Lys Thr Lys Lys Ala
260 265 270

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tcagggtcga tcaggttatt ctgaaaaagg ctgcgtctgt gagcagtttg caaaa atg 178
Met
1

gcc ctc gtt acc gac ttt ctg aac ttt ctg ggc acg aca tgg agc aag 226
Ala Leu Val Thr Asp Phe Leu Asn Phe Leu Gly Thr Thr Trp Ser Lys
5 10 15

tac agc gtg tac acc cat agc tat gct gga aac tat ggg cct act ttg 274
Tyr Ser Val Tyr Thr His Ser Tyr Ala Gly Asn Tyr Gly Pro Thr Leu
20 25 30

aag cac gcc aaa aag gtt tct gct caa ggt aaa act gcg gga cag aca 322
Lys His Ala Lys Lys Val Ser Ala Gln Gly Lys Thr Ala Gly Gln Thr
35 40 45

ctg aga cag aga tcg gtg cag gac aaa aag cca ggc act tac tct ctg 370
Leu Arg Gln Arg Ser Val Gln Asp Lys Lys Pro Gly Thr Tyr Ser Leu
50 55 60 65

gcc gat gtt gct tct cac gac agg cct gga gac tgc tgg atg atc gtc 418
Ala Asp Val Ala Ser His Asp Arg Pro Gly Asp Cys Trp Met Ile Val
70 75 80

aaa gag aag gtg tat gat att agc cgt ttt gcg gac gac cac cct gga 466
Lys Glu Lys Val Tyr Asp Ile Ser Arg Phe Ala Asp Asp His Pro Gly
85 90 95

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ggg acg gta att agc acc tac ttt ggg cgg gat ggc aca gac gtt ttc   514
Gly Thr Val Ile Ser Thr Tyr Phe Gly Arg Asp Gly Thr Asp Val Phe
        100                 105                 110

gca aca ttc cat cca cct gcc gca tgg aag caa ctc aat gac tac tac   562
Ala Thr Phe His Pro Pro Ala Ala Trp Lys Gln Leu Asn Asp Tyr Tyr
    115                 120                 125

att gga gac ctt gct agg gaa gag ccc ctt gat gaa ttg ctt aaa gac   610
Ile Gly Asp Leu Ala Arg Glu Glu Pro Leu Asp Glu Leu Leu Lys Asp
130                 135                 140                 145

tac aga gat atg aga gcc gag ttt gtt aga gaa ggg ctt ttc aag agt   658
Tyr Arg Asp Met Arg Ala Glu Phe Val Arg Glu Gly Leu Phe Lys Ser
                150                 155                 160

tcc aag gcc tgg ttc ctg ctt cag act ctg att aat gca gct ctc ttt   706
Ser Lys Ala Trp Phe Leu Leu Gln Thr Leu Ile Asn Ala Ala Leu Phe
            165                 170                 175

gct gcg agc att gcg act atc tgt tac gac aag agt tac tgg gct att   754
Ala Ala Ser Ile Ala Thr Ile Cys Tyr Asp Lys Ser Tyr Trp Ala Ile
        180                 185                 190

gtg ctg tca gcc agt ttg atg ggt ctc ttc gtc caa cag tgt gga tgg   802
Val Leu Ser Ala Ser Leu Met Gly Leu Phe Val Gln Gln Cys Gly Trp
    195                 200                 205

ctt gcc cat gat ttc ctt cat caa cag gtc ttt gag aac cgt acc gcg   850
Leu Ala His Asp Phe Leu His Gln Gln Val Phe Glu Asn Arg Thr Ala
210                 215                 220                 225

aac tcc ttc ttt ggc tat ttg ttc ggc aat tgc gtg ctt ggc ttt agt   898
Asn Ser Phe Phe Gly Tyr Leu Phe Gly Asn Cys Val Leu Gly Phe Ser
                230                 235                 240

gta tca tgg tgg agg acg aag cac aac att cat cat act gct ccg aat   946
Val Ser Trp Trp Arg Thr Lys His Asn Ile His His Thr Ala Pro Asn
            245                 250                 255
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gag tgc gac gaa cag tac aca cct cta gac gaa gac att gat act ctc   994
Glu Cys Asp Glu Gln Tyr Thr Pro Leu Asp Glu Asp Ile Asp Thr Leu
        260                 265                 270

ccc atc att gcc tgg agc aag gaa att ttg gcc acc gtt gag agc aag  1042
Pro Ile Ile Ala Trp Ser Lys Glu Ile Leu Ala Thr Val Glu Ser Lys
    275                 280                 285

aga att ttg cga gtg ctt caa tat cag cac tac atg att ctg cct cta  1090
Arg Ile Leu Arg Val Leu Gln Tyr Gln His Tyr Met Ile Leu Pro Leu
290                 295                 300                 305

ttg ttc atg gcc cgg tac agt tgg act ttt gga agt ttg ctc ttc aca  1138
Leu Phe Met Ala Arg Tyr Ser Trp Thr Phe Gly Ser Leu Leu Phe Thr
                310                 315                 320

ttc aat cct gat ttg agc acg acc aag gga ttg ata gag aag gga aca  1186
Phe Asn Pro Asp Leu Ser Thr Thr Lys Gly Leu Ile Glu Lys Gly Thr
            325                 330                 335

gtt gct ttt cac tac gcc tgg ttc agt tgg gct gcg ttc cat att ttg  1234
Val Ala Phe His Tyr Ala Trp Phe Ser Trp Ala Ala Phe His Ile Leu
        340                 345                 350

ccg ggt gtc gct aag cct ctt gcg tgg atg gta gca act gag ctt gtg  1282
Pro Gly Val Ala Lys Pro Leu Ala Trp Met Val Ala Thr Glu Leu Val
    355                 360                 365

gcc ggt ttg ttg ttg gga ttc gtg ttt acg ttg agt cac aat gga aag  1330
Ala Gly Leu Leu Leu Gly Phe Val Phe Thr Leu Ser His Asn Gly Lys
370                 375                 380                 385

gag gtt tac aat gaa tcg aag gac ttc gtg aga gcc cag gtt att acc  1378
Glu Val Tyr Asn Glu Ser Lys Asp Phe Val Arg Ala Gln Val Ile Thr
                390                 395                 400

acc cgt aac acc aag cga ggc tgg ttc aac gat tgg ttc act ggg gga  1426
Thr Arg Asn Thr Lys Arg Gly Trp Phe Asn Asp Trp Phe Thr Gly Gly
            405                 410                 415
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ctc gac acc cag att gag cat cac ctg ttt cca aca atg ccc agg cac 1474
Leu Asp Thr Gln Ile Glu His His Leu Phe Pro Thr Met Pro Arg His
420 425 430

aac tac ccc aag atc gca cct cag gtc gag gct ctt tgc aag aag cac 1522
Asn Tyr Pro Lys Ile Ala Pro Gln Val Glu Ala Leu Cys Lys Lys His
435 440 445

ggc ctc gag tac gat aat gtc tcc gtc gtt ggt gcc tct gtc gcg gtt 1570
Gly Leu Glu Tyr Asp Asn Val Ser Val Val Gly Ala Ser Val Ala Val
450 455 460 465

gtg aag gcg ctc aag gaa att gct gat gaa gcg tca att cgg ctt cac 1618
Val Lys Ala Leu Lys Glu Ile Ala Asp Glu Ala Ser Ile Arg Leu His
470 475 480

gct cac taa gaaatcgtcg aactttgact attcatttt ttcgcctggc 1667
Ala His

tacctcaaat gttcgggagc aggtgcttgg cagtgtgttc aaccggagcg cactgaaaat 1727

gtgcagaatc catttccaga aattaccatt cctagctaaa tcttctttt accaggtcgg 1787

atatatgaaa ctttttgat gcaacaagta gcattcaatt gaagacattg ttcgagatat 1847

aattcgcagt gtttctattc agcgggcata cgtactagtc catatcggcg gttgccgaga 1907

gtttacatta ttagttggca caacgagtag atctagtgta aatttctatt tccgcatgta 1967

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            20                  25                  30

Leu Lys His Ala Lys Lys Val Ser Ala Gln Gly Lys Thr Ala Gly Gln
        35                  40                  45

Thr Leu Arg Gln Arg Ser Val Gln Asp Lys Lys Pro Gly Thr Tyr Ser
    50                  55                  60

Leu Ala Asp Val Ala Ser His Asp Arg Pro Gly Asp Cys Trp Met Ile
65                  70                  75                  80

Val Lys Glu Lys Val Tyr Asp Ile Ser Arg Phe Ala Asp Asp His Pro
                85                  90                  95

Gly Gly Thr Val Ile Ser Thr Tyr Phe Gly Arg Asp Gly Thr Asp Val
            100                 105                 110

Phe Ala Thr Phe His Pro Pro Ala Ala Trp Lys Glu Leu Asn Asp Tyr
        115                 120                 125

Tyr Ile Gly Asp Leu Ala Arg Glu Glu Pro Leu Asp Glu Leu Leu Lys
    130                 135                 140

Asp Tyr Arg Asp Met Arg Ala Glu Phe Val Arg Glu Gly Leu Phe Lys
145                 150                 155                 160

Ser Ser Lys Ala Trp Phe Leu Leu Gln Thr Leu Ile Asn Ala Ala Leu
                165                 170                 175

Phe Ala Ala Ser Ile Ala Thr Ile Cys Tyr Asp Lys Ser Tyr Trp Ala
            180                 185                 190

Ile Val Leu Ser Ala Ser Leu Met Gly Leu Phe Val Gln Gln Cys Gly
        195                 200                 205
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Trp Leu Ala His Asp Phe Leu His Gln Gln Val Phe Glu Asn Arg Thr
210 215 220

Ala Asn Ser Phe Phe Gly Tyr Leu Phe Gly Asn Cys Val Leu Gly Phe
225 230 235 240

Ser Val Ser Trp Trp Arg Thr Lys His Asn Ile His His Thr Ala Pro
245 250 255

Asn Glu Cys Asp Glu Gln Tyr Thr Pro Leu Asp Glu Asp Ile Asp Thr
260 265 270

Leu Pro Ile Ile Ala Trp Ser Lys Glu Ile Leu Ala Thr Val Glu Ser
275 280 285

Lys Arg Ile Leu Arg Val Leu Gln Tyr Gln His Tyr Met Ile Leu Pro
290 295 300

Leu Leu Phe Met Ala Arg Tyr Ser Trp Thr Phe Gly Ser Leu Leu Phe
305 310 315 320

Thr Phe Asn Pro Asp Leu Ser Thr Thr Lys Gly Leu Ile Glu Lys Gly
325 330 335

Thr Val Ala Phe His Tyr Ala Trp Phe Ser Trp Ala Ala Phe His Ile
340 345 350

Leu Pro Gly Val Ala Lys Pro Leu Ala Trp Met Val Ala Thr Glu Leu
355 360 365

Val Ala Gly Leu Leu Leu Gly Phe Val Phe Thr Leu Ser His Asn Gly
370 375 380

Lys Glu Val Tyr Asn Glu Ser Lys Asp Phe Val Arg Ala Gln Val Ile
385 390 395 400

Thr Thr Arg Asn Thr Lys Arg Gly Trp Phe Asn Asp Trp Phe Thr Gly
405 410 415

Gly Leu Asp Thr Gln Ile Glu His His Leu Phe Pro Thr Met Pro Arg

420 425 430

His Asn Tyr Pro Lys Ile Ala Pro Gln Val Glu Ala Leu Cys Lys Lys
435 440 445

His Gly Leu Glu Tyr Asp Asn Val Ser Val Val Gly Ala Ser Val Ala
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Val Val Lys Ala Leu Lys Glu Ile Ala Asp Glu Ala Ser Ile Arg Leu
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tgg agc aag tac agc gtg tac acc cat agc tat gct gga aac tat ggg 99
Trp Ser Lys Tyr Ser Val Tyr Thr His Ser Tyr Ala Gly Asn Tyr Gly
15 20 25 30

cct act ttg aag cac gcc aaa aag gtt tct gct caa ggt aaa act gcg 147
Pro Thr Leu Lys His Ala Lys Lys Val Ser Ala Gln Gly Lys Thr Ala
35 40 45

gga cag aca ctg aga cag aga tcg gtg cag gac aaa aag cca ggc act 195
Gly Gln Thr Leu Arg Gln Arg Ser Val Gln Asp Lys Lys Pro Gly Thr
50 55 60

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tac tct ctg gcc gat gtt gct tct cac gac agg cct gga gac tgc tgg   243
Tyr Ser Leu Ala Asp Val Ala Ser His Asp Arg Pro Gly Asp Cys Trp
        65                  70                  75

atg atc gtc aaa gag aag gtg tat gat att agc cgt ttt gcg gac gac   291
Met Ile Val Lys Glu Lys Val Tyr Asp Ile Ser Arg Phe Ala Asp Asp
    80                  85                  90

cac cct gga ggg acg gta att agc acc tac ttt ggg cgg gat ggc aca   339
His Pro Gly Gly Thr Val Ile Ser Thr Tyr Phe Gly Arg Asp Gly Thr
95                  100                 105                 110

gac gtt ttc gca aca ttc cat cca cct gcc gca tgg aag caa ctc aat   387
Asp Val Phe Ala Thr Phe His Pro Pro Ala Ala Trp Lys Gln Leu Asn
                115                 120                 125

gac tac tac att gga gac ctt gct agg gaa gag ccc ctt gat gaa ttg   435
Asp Tyr Tyr Ile Gly Asp Leu Ala Arg Glu Glu Pro Leu Asp Glu Leu
            130                 135                 140

ctt aaa gac tac aga gat atg aga gcc gag ttt gtt aga gaa ggg ctt   483
Leu Lys Asp Tyr Arg Asp Met Arg Ala Glu Phe Val Arg Glu Gly Leu
        145                 150                 155

ttc aag agt tcc aag gcc tgg ttc ctg ctt cag act ctg att aat gca   531
Phe Lys Ser Ser Lys Ala Trp Phe Leu Leu Gln Thr Leu Ile Asn Ala
    160                 165                 170

gct ctc ttt gct gcg agc att gcg act atc tgt tac gac aag agt tac   579
Ala Leu Phe Ala Ala Ser Ile Ala Thr Ile Cys Tyr Asp Lys Ser Tyr
175                 180                 185                 190

tgg gct att gtg ctg tca gcc agt ttg atg ggt ctc ttc gtc caa cag   627
Trp Ala Ile Val Leu Ser Ala Ser Leu Met Gly Leu Phe Val Gln Gln
                195                 200                 205

tgt gga tgg ctt gcc cat gat ttc ctt cat caa cag gtc ttt gag aac   675
Cys Gly Trp Leu Ala His Asp Phe Leu His Gln Gln Val Phe Glu Asn
            210                 215                 220
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cgt acc gcg aac tcc ttc ttt ggc tat ttg ttc ggc aat tgc gtg ctt   723
Arg Thr Ala Asn Ser Phe Phe Gly Tyr Leu Phe Gly Asn Cys Val Leu
        225                 230                 235

ggc ttt agt gta tca tgg tgg agg acg aag cac aac att cat cat act   771
Gly Phe Ser Val Ser Trp Trp Arg Thr Lys His Asn Ile His His Thr
    240                 245                 250

gct ccg aat gag tgc gac gaa cag tac aca cct cta gac gaa gac att   819
Ala Pro Asn Glu Cys Asp Glu Gln Tyr Thr Pro Leu Asp Glu Asp Ile
255                 260                 265                 270

gat act ctc ccc atc att gcc tgg agc aag gaa att ttg gcc acc gtt   867
Asp Thr Leu Pro Ile Ile Ala Trp Ser Lys Glu Ile Leu Ala Thr Val
                275                 280                 285

gag agc aag aga att ttg cga gtg ctt caa tat cag cac tac atg att   915
Glu Ser Lys Arg Ile Leu Arg Val Leu Gln Tyr Gln His Tyr Met Ile
            290                 295                 300

ctg cct cta ttg ttc atg gcc cgg tac agt tgg act ttt gga agt ttg   963
Leu Pro Leu Leu Phe Met Ala Arg Tyr Ser Trp Thr Phe Gly Ser Leu
        305                 310                 315

ctc ttc aca ttc aat cct gat ttg agc acg acc aag gga ttg ata gag  1011
Leu Phe Thr Phe Asn Pro Asp Leu Ser Thr Thr Lys Gly Leu Ile Glu
    320                 325                 330

aag gga aca gtt gct ttt cac tac gcc tgg ttc agt tgg gct gcg ttc  1059
Lys Gly Thr Val Ala Phe His Tyr Ala Trp Phe Ser Trp Ala Ala Phe
335                 340                 345                 350

cat att ttg ccg ggt gtc gct aag cct ctt gcg tgg atg gta gca act  1107
His Ile Leu Pro Gly Val Ala Lys Pro Leu Ala Trp Met Val Ala Thr
                355                 360                 365

gag ctt gtg gcc ggt ttg ttg ttg gga ttc gtg ttt acg ttg agt cac  1155
Glu Leu Val Ala Gly Leu Leu Leu Gly Phe Val Phe Thr Leu Ser His
            370                 375                 380
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aat gga aag gag gtt tac aat gaa tcg aag gac ttc gtg aga gcc cag   1203
Asn Gly Lys Glu Val Tyr Asn Glu Ser Lys Asp Phe Val Arg Ala Gln
        385                 390                 395

gtt att acc acc cgt aac acc aag cga ggc tgg ttc aac gat tgg ttc   1251
Val Ile Thr Thr Arg Asn Thr Lys Arg Gly Trp Phe Asn Asp Trp Phe
    400                 405                 410

act ggg gga ctc gac acc cag att gag cat cac ctg ttt cca aca atg   1299
Thr Gly Gly Leu Asp Thr Gln Ile Glu His His Leu Phe Pro Thr Met
415                 420                 425                 430

ccc agg cac aac tac ccc aag atc gca cct cag gtc gag gct ctt tgc   1347
Pro Arg His Asn Tyr Pro Lys Ile Ala Pro Gln Val Glu Ala Leu Cys
                435                 440                 445

aag aag cac ggc ctc gag tac gat aat gtc tcc gtc gtt ggt gcc tct   1395
Lys Lys His Gly Leu Glu Tyr Asp Asn Val Ser Val Val Gly Ala Ser
            450                 455                 460

gtc gcg gtt gtg aag gcg ctc aag gaa att gct gat gaa gcg tca att   1443
Val Ala Val Val Lys Ala Leu Lys Glu Ile Ala Asp Glu Ala Ser Ile
        465                 470                 475

cgg ctt cac gct cac taa gtcgac                                   1467
Arg Leu His Ala His
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<212> PRT
<213> Ceratodon purpureus

<400> 10

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Lys Tyr Ser Val Tyr Thr His Ser Tyr Ala Gly Asn Tyr Gly Pro Thr
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            20                  25                  30

Leu Lys His Ala Lys Lys Val Ser Ala Gln Gly Lys Thr Ala Gly Gln
        35                  40                  45

Thr Leu Arg Gln Arg Ser Val Gln Asp Lys Lys Pro Gly Thr Tyr Ser
    50                  55                  60

Leu Ala Asp Val Ala Ser His Asp Arg Pro Gly Asp Cys Trp Met Ile
65                  70                  75                  80

Val Lys Glu Lys Val Tyr Asp Ile Ser Arg Phe Ala Asp Asp His Pro
                85                  90                  95

Gly Gly Thr Val Ile Ser Thr Tyr Phe Gly Arg Asp Gly Thr Asp Val
            100                 105                 110

Phe Ala Thr Phe His Pro Pro Ala Ala Trp Lys Gln Leu Asn Asp Tyr
        115                 120                 125

Tyr Ile Gly Asp Leu Ala Arg Glu Glu Pro Leu Asp Glu Leu Leu Lys
    130                 135                 140

Asp Tyr Arg Asp Met Arg Ala Glu Phe Val Arg Glu Gly Leu Phe Lys
145                 150                 155                 160

Ser Ser Lys Ala Trp Phe Leu Leu Gln Thr Leu Ile Asn Ala Ala Leu
                165                 170                 175

Phe Ala Ala Ser Ile Ala Thr Ile Cys Tyr Asp Lys Ser Tyr Trp Ala
            180                 185                 190

Ile Val Leu Ser Ala Ser Leu Met Gly Leu Phe Val Gln Gln Cys Gly
        195                 200                 205

Trp Leu Ala His Asp Phe Leu His Gln Gln Val Phe Glu Asn Arg Thr
    210                 215                 220

Ala Asn Ser Phe Phe Gly Tyr Leu Phe Gly Asn Cys Val Leu Gly Phe
225                 230                 235                 240
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Ser Val Ser Trp Trp Arg Thr Lys His Asn Ile His His Thr Ala Pro
245 250 255

Asn Glu Cys Asp Glu Gln Tyr Thr Pro Leu Asp Glu Asp Ile Asp Thr
260 265 270

Leu Pro Ile Ile Ala Trp Ser Lys Glu Ile Leu Ala Thr Val Glu Ser
275 280 285

Lys Arg Ile Leu Arg Val Leu Gln Tyr Gln His Tyr Met Ile Leu Pro
290 295 300

Leu Leu Phe Met Ala Arg Tyr Ser Trp Thr Phe Gly Ser Leu Leu Phe
305 310 315 320

Thr Phe Asn Pro Asp Leu Ser Thr Thr Lys Gly Leu Ile Glu Lys Gly
325 330 335

Thr Val Ala Phe His Tyr Ala Trp Phe Ser Trp Ala Ala Phe His Ile
340 345 350

Leu Pro Gly Val Ala Lys Pro Leu Ala Trp Met Val Ala Thr Glu Leu
355 360 365

Val Ala Gly Leu Leu Leu Gly Phe Val Phe Thr Leu Ser His Asn Gly
370 375 380

Lys Glu Val Tyr Asn Glu Ser Lys Asp Phe Val Arg Ala Gln Val Ile
385 390 395 400

Thr Thr Arg Asn Thr Lys Arg Gly Trp Phe Asn Asp Trp Phe Thr Gly
405 410 415

Gly Leu Asp Thr Gln Ile Glu His His Leu Phe Pro Thr Met Pro Arg
420 425 430

His Asn Tyr Pro Lys Ile Ala Pro Gln Val Glu Ala Leu Cys Lys Lys
435 440 445

His Gly Leu Glu Tyr Asp Asn Val Ser Val Val Gly Ala Ser Val Ala
450 455 460

Val Val Lys Ala Leu Lys Glu Ile Ala Asp Glu Ala Ser Ile Arg Leu
465 470 475 480

His Ala His

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<213> Ceratodon purpureus

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<223> Δ6-desaturase

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tttcgtggtt ttggcgttgg cattgcgcga gggcggacag tgggagtgcg gyaggtctgt 120

ttgtgcatga cgaggtggtt gtaatcttcg cgggcaga atg gtg tcc cag ggc ggc 176
Met Val Ser Gln Gly Gly
1 5

ggt ctc tcg cag ggt tcc att gaa gaa aac att gac gtt gag cac ttg 224
Gly Leu Ser Gln Gly Ser Ile Glu Glu Asn Ile Asp Val Glu His Leu
10 15 20

gca acg atg ccc ctc gtc agt gac ttc cta aat gtc ctg gga acg act 272
Ala Thr Met Pro Leu Val Ser Asp Phe Leu Asn Val Leu Gly Thr Thr
25 30 35

ttg ggc cag tgg agt ctt tcc act aca ttc gct ttc aag agg ctc acg 320
Leu Gly Gln Trp Ser Leu Ser Thr Thr Phe Ala Phe Lys Arg Leu Thr
40 45 50

act aag aaa cac agt tcg gac atc tcg gtg gag gca caa aaa gaa tcg 368
Thr Lys Lys His Ser Ser Asp Ile Ser Val Glu Ala Gln Lys Glu Ser
55 60 65 70

gtt gcg cgg ggg cca gtt gag aat att tct caa tcg gtt gcg cag ccc 416
Val Ala Arg Gly Pro Val Glu Asn Ile Ser Gln Ser Val Ala Gln Pro
75 80 85

atc agg cgg agg tgg gtg cag gat aaa aag ccg gtt act tac agc ctg 464
Ile Arg Arg Arg Trp Val Gln Asp Lys Lys Pro Val Thr Tyr Ser Leu
90 95 100

aag gat gta gct tcg cac gat atg ccc cag gac tgc tgg att ata atc 512
Lys Asp Val Ala Ser His Asp Met Pro Gln Asp Cys Trp Ile Ile Ile
105 110 115

aaa gag aag gtg tat gat gtg agc aac ttc gct gag cag cac cct gga 560
Lys Glu Lys Val Tyr Asp Val Ser Thr Phe Ala Glu Gln His Pro Gly
120 125 130

ggc acg gtt atc aac acc tac ttc gga cga gac gcc aca gat gtt ttc 608
Gly Thr Val Ile Asn Thr Tyr Phe Gly Arg Asp Ala Thr Asp Val Phe
135 140 145 150

tct act ttc cac gca tcc agc tca tgg aag att ctt cag aat ttc tac 656
Ser Thr Phe His Ala Ser Thr Ser Trp Lys Ile Leu Gln Asn Phe Tyr
155 160 165

atc ggg aac ctt gtt agg gag gag ccg act ttg gag ctg ctg aag gag 704
Ile Gly Asn Leu Val Arg Glu Glu Pro Thr Leu Glu Leu Leu Lys Glu
170 175 180

tac aga gag ttg aga gcc ctt ttc ttg aga gaa cag ctt ttc aag agt 752
Tyr Arg Glu Leu Arg Ala Leu Phe Leu Arg Glu Gln Leu Phe Lys Ser
185 190 195

tcc aaa tcc tac tac ctt ttc aag act ctc ata aat gtt tcc att gtt 800
Ser Lys Ser Tyr Tyr Leu Phe Lys Thr Leu Ile Asn Val Ser Ile Val
200 205 210

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gcc aca agc att gcg ata atc agt ctg tac aag tct tac cgg gcg gtt   848
Ala Thr Ser Ile Ala Ile Ile Ser Leu Tyr Lys Ser Tyr Arg Ala Val
215                 220                 225                 230

ctg tta tca gcc agt ttg atg ggc ttg ttt att caa cag tgc gga tgg   896
Leu Leu Ser Ala Ser Leu Met Gly Leu Phe Ile Gln Gln Cys Gly Trp
                235                 240                 245

ttg tct cac gat ttt cta cac cat cag gta ttt gag aca cgc tgg ctc   944
Leu Ser His Asp Phe Leu His His Gln Val Phe Glu Thr Arg Trp Leu
            250                 255                 260

aat gac gtt gtt ggc tat gtg gtc ggc aac gtt gtt ctg gga ttc agt   992
Asn Asp Val Val Gly Tyr Val Val Gly Asn Val Val Leu Gly Phe Ser
        265                 270                 275

gtc tcg tgg tgg aag acc aag cac aac ctg cat cat gct gct ccg aat   1040
Val Ser Trp Trp Lys Thr Lys His Asn Leu His His Ala Ala Pro Asn
    280                 285                 290

gaa tgc gac caa aag tac aca ccg att gat gag gat att gat act ctc   1088
Glu Cys Asp Gln Lys Tyr Thr Pro Ile Asp Glu Asp Ile Asp Thr Leu
295                 300                 305                 310

ccc atc att gct tgg agt aaa gat ctc ttg gcc act gtt gag agc aag   1136
Pro Ile Ile Ala Trp Ser Lys Asp Leu Leu Ala Thr Val Glu Ser Lys
                315                 320                 325

acc atg ttg cga gtt ctt cag tac cag cac cta ttc ttt ttg gtt ctt   1184
Thr Met Leu Arg Val Leu Gln Tyr Gln His Leu Phe Phe Leu Val Leu
            330                 335                 340

ttg acg ttt gcc cgg gcg agt tgg cta ttt tgg agc gcg gcc ttc act   1232
Leu Thr Phe Ala Arg Ala Ser Trp Leu Phe Trp Ser Ala Ala Phe Thr
        345                 350                 355

ctc agg ccc gag ttg acc ctt ggc gag aag ctt ttg gag agg gga acg   1280
Leu Arg Pro Glu Leu Thr Leu Gly Glu Lys Leu Leu Glu Arg Gly Thr
    360                 365                 370
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atg gct ttg cac tac att tgg ttt aat agt gtt gcg ttt tat ctg ctc 1328
Met Ala Leu His Tyr Ile Trp Phe Asn Ser Val Ala Phe Tyr Leu Leu
375 380 385 390

ccc gga tgg aaa cca gtt gta tgg atg gtg gtc agc gag ctc atg tct 1376
Pro Gly Trp Lys Pro Val Val Trp Met Val Val Ser Glu Leu Met Ser
395 400 405

ggt ttc ctg ctg gga tac gta ttt gta ctc agt cac aat gga atg gag 1424
Gly Phe Leu Leu Gly Tyr Val Phe Val Leu Ser His Asn Gly Met Glu
410 415 420

gtg tac aat acg tca aag gac ttc gtg aat gcc cag att gca tcg act 1472
Val Tyr Asn Thr Ser Lys Asp Phe Val Asn Ala Gln Ile Ala Ser Thr
425 430 435

cgc gac atc aaa gca ggg gtg ttt aat gat tgg ttc acc gga ggt ctc 1520
Arg Asp Ile Lys Ala Gly Val Phe Asn Asp Trp Phe Thr Gly Gly Leu
440 445 450

aac aga cag att gag cat cat cta ttt cca acg atg ccc agg cac aac 1568
Asn Arg Gln Ile Glu His His Leu Phe Pro Thr Met Pro Arg His Asn
455 460 465 470

ctt aat aaa att tct cct cac gtg gag act ttg tgc aaa aag cat gga 1616
Leu Asn Lys Ile Ser Pro His Val Glu Thr Leu Cys Lys Lys His Gly
475 480 485

ctg gtc tac gaa gac gtg agc atg gct tcg ggc act tac cgg gtt ttg 1664
Leu Val Tyr Glu Asp Val Ser Met Ala Ser Gly Thr Tyr Arg Val Leu
490 495 500

aaa aca ctt aag gac gtt gcc gat gct gct tca cac cag cag ctt gct 1712
Lys Thr Leu Lys Asp Val Ala Asp Ala Ala Ser His Gln Gln Leu Ala
505 510 515

gcg agt tga ggcatcgcag cactcgtcga aacatttttg tctgttatag 1761
Ala Ser
520

tgttcatatg tgatcgaggg gaaaaggtcc catgctctga tctattcttc tgtagccaat 1821

atttttcaat tgaaaggagg ttcctcactt atcttccatc tatcgttgca catcctgcat 1881

cagagttagc gttggagtaa tgttaagcac ttgtagatta tgcccaccat tgccacattt 1941

ctgttcggtt acaatcgttt gattccatgc tatcctccgt gttcatctcg ttgttataag 2001

caagcttgaa aaaacatgct acgagattgg cagacgttgt cttggcagct gtagaggttg 2061

gttccattca ttgtgtagta cagaactctc tcgtcccttt ttctctacat tacttgttac 2121

atagtgactt tcattcacag caaaaaaaaa aaaaaaaaa 2160

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<213> Ceratodon purpureus

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1 5 10 15

Ile Asp Val Glu His Leu Ala Thr Met Pro Leu Val Ser Asp Phe Leu
20 25 30

Asn Val Leu Gly Thr Thr Leu Gly Gln Trp Ser Leu Ser Thr Thr Phe
35 40 45

Ala Phe Lys Arg Leu Thr Thr Lys Lys His Ser Ser Asp Ile Ser Val
50 55 60

Glu Ala Gln Lys Glu Ser Val Ala Arg Gly Pro Val Glu Asn Ile Ser
65 70 75 80

Gln Ser Val Ala Gln Pro Ile Arg Arg Arg Trp Val Gln Asp Lys Lys
85 90 95

Pro Val Thr Tyr Ser Leu Lys Asp Val Ala Ser His Asp Met Pro Gln

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          100                 105                 110

Asp Cys Trp Ile Ile Ile Lys Glu Lys Val Tyr Asp Val Ser Thr Phe
        115                 120                 125

Ala Glu Gln His Pro Gly Gly Thr Val Ile Asn Thr Tyr Phe Gly Arg
    130                 135                 140

Asp Ala Thr Asp Val Phe Ser Thr Phe His Ala Ser Thr Ser Trp Lys
145                 150                 155                 160

Ile Leu Gln Asn Phe Tyr Ile Gly Asn Leu Val Arg Glu Glu Pro Thr
                165                 170                 175

Leu Glu Leu Leu Lys Glu Tyr Arg Glu Leu Arg Ala Leu Phe Leu Arg
            180                 185                 190

Glu Gln Leu Phe Lys Ser Ser Lys Ser Tyr Tyr Leu Phe Lys Thr Leu
        195                 200                 205

Ile Asn Val Ser Ile Val Ala Thr Ser Ile Ala Ile Ile Ser Leu Tyr
    210                 215                 220

Lys Ser Tyr Arg Ala Val Leu Leu Ser Ala Ser Leu Met Gly Leu Phe
225                 230                 235                 240

Ile Gln Gln Cys Gly Trp Leu Ser His Asp Phe Leu His His Gln Val
                245                 250                 255

Phe Glu Thr Arg Trp Leu Asn Asp Val Val Gly Tyr Val Val Gly Asn
            260                 265                 270

Val Val Leu Gly Phe Ser Val Ser Trp Trp Lys Thr Lys His Asn Leu
        275                 280                 285

His His Ala Ala Pro Asn Glu Cys Asp Gln Lys Tyr Thr Pro Ile Asp
    290                 295                 300

Glu Asp Ile Asp Thr Leu Pro Ile Ile Ala Trp Ser Lys Asp Leu Leu
305                 310                 315                 320
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Ala Thr Val Glu Ser Lys Thr Met Leu Arg Val Leu Gln Tyr Gln His
325 330 335

Leu Phe Phe Leu Val Leu Leu Thr Phe Ala Arg Ala Ser Trp Leu Phe
340 345 350

Trp Ser Ala Ala Phe Thr Leu Arg Pro Glu Leu Thr Leu Gly Glu Lys
355 360 365

Leu Leu Glu Arg Gly Thr Met Ala Leu His Tyr Ile Trp Phe Asn Ser
370 375 380

Val Ala Phe Tyr Leu Leu Pro Gly Trp Lys Pro Val Val Trp Met Val
385 390 395 400

Val Ser Glu Leu Met Ser Gly Phe Leu Leu Gly Tyr Val Phe Val Leu
405 410 415

Ser His Asn Gly Met Glu Val Tyr Asn Thr Ser Lys Asp Phe Val Asn
420 425 430

Ala Gln Ile Ala Ser Thr Arg Asp Ile Lys Ala Gly Val Phe Asn Asp
435 440 445

Trp Phe Thr Gly Gly Leu Asn Arg Gln Ile Glu His His Leu Phe Pro
450 455 460

Thr Met Pro Arg His Asn Leu Asn Lys Ile Ser Pro His Val Glu Thr
465 470 475 480

Leu Cys Lys Lys His Gly Leu Val Tyr Glu Asp Val Ser Met Ala Ser
485 490 495

Gly Thr Tyr Arg Val Leu Lys Thr Leu Lys Asp Val Ala Asp Ala Ala
500 505 510

Ser His Gln Gln Leu Ala Ala Ser
515 520

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<212> DNA
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<220>
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<223> Δ6-desaturase

<400> 13

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Met Gly Lys Gly Gly Asp Ala Arg Ala Ser Lys Gly Ser Thr Ala Ala
  1               5                  10                  15

cgc aag atc agt tgg cag gaa gtc aag acc cac gcg tct ccg gag gac   96
Arg Lys Ile Ser Trp Gln Glu Val Lys Thr His Ala Ser Pro Glu Asp
            20                  25                  30

gcc tgg atc att cac tcc aat aag gtc tac gac gtg tcc aac tgg cac   144
Ala Trp Ile Ile His Ser Asn Lys Val Tyr Asp Val Ser Asn Trp His
        35                  40                  45

gaa cat ccc gga ggc gcc gtc att ttc acg cac qcc qqt gac gac atg   192
Glu His Pro Gly Gly Ala Val Ile Phe Thr His Ala Gly Asp Asp Met
    50                  55                  60

acg gac att ttc gct gcc ttt cac gca ccc gga tcg cag tcg ctc atg   240
Thr Asp Ile Phe Ala Ala Phe His Ala Pro Gly Ser Gln Ser Leu Met
65                  70                  75                  80

aag aag ttc tac att ggc gaa ttg ctc ccg gaa acc acc ggc aag gag   288
Lys Lys Phe Tyr Ile Gly Glu Leu Leu Pro Glu Thr Thr Gly Lys Glu
                85                  90                  95

ccg cag caa atc gcc ttt gaa aag ggc tac cgc gat ctg cgc tcc aaa   336
Pro Gln Gln Ile Ala Phe Glu Lys Gly Tyr Arg Asp Leu Arg Ser Lys
            100                 105                 110
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ctc atc atg atg ggc atg ttc aag tcc aac aag tgg ttc tac gtc tac   384
Leu Ile Met Met Gly Met Phe Lys Ser Asn Lys Trp Phe Tyr Val Tyr
        115                 120                 125

aag tgc ctc agc aac atg gcc att tgg gcc gcc gcc tgt gct ctc gtc   432
Lys Cys Leu Ser Asn Met Ala Ile Trp Ala Ala Ala Cys Ala Leu Val
    130                 135                 140

ttt tac tcg gac cgc ttc tgg gta cac ctg gcc agc gcc gtc atg ctg   480
Phe Tyr Ser Asp Arg Phe Trp Val His Leu Ala Ser Ala Val Met Leu
145                 150                 155                 160

gga aca ttc ttt cag cag tcg gga tgg ttg gca cac gac ttt ctg cac   528
Gly Thr Phe Phe Gln Gln Ser Gly Trp Leu Ala His Asp Phe Leu His
                165                 170                 175

cac cag gtc ttc acc aag cgc aag cac ggg gat ctc gga gga ctc ttt   576
His Gln Val Phe Thr Lys Arg Lys His Gly Asp Leu Gly Gly Leu Phe
            180                 185                 190

tgg ggg aac ctc atg cag ggt tac tcc gta cag tgg tgg aaa aac aag   624
Trp Gly Asn Leu Met Gln Gly Tyr Ser Val Gln Trp Trp Lys Asn Lys
        195                 200                 205

cac aac gga cac cac gcc gtc ccc aac ctc cac tgc tcc tcc gca gtc   672
His Asn Gly His His Ala Val Pro Asn Leu His Cys Ser Ser Ala Val
    210                 215                 220

gcg caa gat ggg gac ccg gac atc gat acc atg ccc ctt ctc gcc tgg   720
Ala Gln Asp Gly Asp Pro Asp Ile Asp Thr Met Pro Leu Leu Ala Trp
225                 230                 235                 240

tcc gtc cag caa gcc cag tct tac cgg gaa ctc caa gcc gac gga aag   768
Ser Val Gln Gln Ala Gln Ser Tyr Arg Glu Leu Gln Ala Asp Gly Lys
                245                 250                 255

gat tcg ggt ttg gtc aag ttc atg atc cgt aac caa tcc tac ttt tac   816
Asp Ser Gly Leu Val Lys Phe Met Ile Arg Asn Gln Ser Tyr Phe Tyr
            260                 265                 270
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ttt ccc atc ttg ttg ctc gcc cgc ctg tcg tgg ttg aac gag tcc ttc   864
Phe Pro Ile Leu Leu Leu Ala Arg Leu Ser Trp Leu Asn Glu Ser Phe
        275                 280                 285

aag tgc gcc ttt ggg ctt gga gct gcg tcg gag aac gct gct ctc gaa   912
Lys Cys Ala Phe Gly Leu Gly Ala Ala Ser Glu Asn Ala Ala Leu Glu
    290                 295                 300

ctc aag gcc aag ggt ctt cag tac ccc ctt ttg gaa aag gct ggc atc   960
Leu Lys Ala Lys Gly Leu Gln Tyr Pro Leu Leu Glu Lys Ala Gly Ile
305                 310                 315                 320

ctg ctg cac tac gct tgg atg ctt aca gtt tcg tcc ggc ttt gga cgc  1008
Leu Leu His Tyr Ala Trp Met Leu Thr Val Ser Ser Gly Phe Gly Arg
                325                 330                 335

ttc tcg ttc gcg tac acc gca ttt tac ttt cta acc gcg acc gcg tcc  1056
Phe Ser Phe Ala Tyr Thr Ala Phe Tyr Phe Leu Thr Ala Thr Ala Ser
            340                 345                 350

tgt gga ttc ttg ctc gcc att gtc ttt ggc ctc ggc cac aac ggc atg  1104
Cys Gly Phe Leu Leu Ala Ile Val Phe Gly Leu Gly His Asn Gly Met
        355                 360                 365

gcc acc tac aat gcc gac gcc cgt ccg gac ttc tgg aag ctc caa gtc  1152
Ala Thr Tyr Asn Ala Asp Ala Arg Pro Asp Phe Trp Lys Leu Gln Val
    370                 375                 380

acc acg act cgc aac gtc acg ggc gga cac ggt ttc ccc caa gcc ttt  1200
Thr Thr Thr Arg Asn Val Thr Gly Gly His Gly Phe Pro Gln Ala Phe
385                 390                 395                 400

gtc gac tgg ttc tgt ggt ggc ctc cag tac caa gtc gac cac cac tta  1248
Val Asp Trp Phe Cys Gly Gly Leu Gln Tyr Gln Val Asp His His Leu
                405                 410                 415

ttc ccc agc ctg ccc cga cac aat ctg gcc aag aca cac gca ctg gtc  1296
Phe Pro Ser Leu Pro Arg His Asn Leu Ala Lys Thr His Ala Leu Val
            420                 425                 430
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gaa tcg ttc tgc aag gag tgg ggt gtc cag tac cac gaa gcc gac ctt 1344
Glu Ser Phe Cys Lys Glu Trp Gly Val Gln Tyr His Glu Ala Asp Leu
435 440 445

gtg gac ggg acc atg gaa gtc ttg cac cat ttg ggc agc gtg gcc ggc 1392
Val Asp Gly Thr Met Glu Val Leu His His Leu Gly Ser Val Ala Gly
450 455 460

gaa ttc gtc gtg gat ttt gta cgc gat gga ccc gcc atg taa 1434
Glu Phe Val Val Asp Phe Val Arg Asp Gly Pro Ala Met
465 470 475

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<213> Phaeodactylum tricornutum

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Met Gly Lys Gly Gly Asp Ala Arg Ala Ser Lys Gly Ser Thr Ala Ala
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Arg Lys Ile Ser Trp Gln Glu Val Lys Thr His Ala Ser Pro Glu Asp
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Ala Trp Ile Ile His Ser Asn Lys Val Tyr Asp Val Ser Asn Trp His
35 40 45

Glu His Pro Gly Gly Ala Val Ile Phe Thr His Ala Gly Asp Asp Met
50 55 60

Thr Asp Ile Phe Ala Ala Phe His Ala Pro Gly Ser Gln Ser Leu Met
65 70 75 80

Lys Lys Phe Tyr Ile Gly Glu Leu Leu Pro Glu Thr Thr Gly Lys Glu
85 90 95

Pro Gln Gln Ile Ala Phe Glu Lys Gly Tyr Arg Asp Leu Arg Ser Lys
100 105 110

Leu Ile Met Met Gly Met Phe Lys Ser Asn Lys Trp Phe Tyr Val Tyr
115 120 125

Lys Cys Leu Ser Asn Met Ala Ile Trp Ala Ala Ala Cys Ala Leu Val
130 135 140

Phe Tyr Ser Asp Arg Phe Trp Val His Leu Ala Ser Ala Val Met Leu
145 150 155 160

Gly Thr Phe Phe Gln Gln Ser Gly Trp Leu Ala His Asp Phe Leu His
165 170 175

His Gln Val Phe Thr Lys Arg Lys His Gly Asp Leu Gly Gly Leu Phe
180 185 190

Trp Gly Asn Leu Met Gln Gly Tyr Ser Val Gln Trp Trp Lys Asn Lys
195 200 205

His Asn Gly His His Ala Val Pro Asn Leu His Cys Ser Ser Ala Val
210 215 220

Ala Gln Asp Gly Asp Pro Asp Ile Asp Thr Met Pro Leu Leu Ala Trp
225 230 235 240

Ser Val Gln Gln Ala Gln Ser Tyr Arg Glu Leu Gln Ala Asp Gly Lys
245 250 255

Asp Ser Gly Leu Val Lys Phe Met Ile Arg Asn Gln Ser Tyr Phe Tyr
260 265 270

Phe Pro Ile Leu Leu Leu Ala Arg Leu Ser Trp Leu Asn Glu Ser Phe
275 280 285

Lys Cys Ala Phe Gly Leu Gly Ala Ala Ser Glu Asn Ala Ala Leu Glu
290 295 300

Leu Lys Ala Lys Gly Leu Gln Tyr Pro Leu Leu Glu Lys Ala Gly Ile
305 310 315 320

Leu Leu His Tyr Ala Trp Met Leu Thr Val Ser Ser Gly Phe Gly Arg

325 330 335

Phe Ser Phe Ala Tyr Thr Ala Phe Tyr Phe Leu Thr Ala Thr Ala Ser
340 345 350

Cys Gly Phe Leu Leu Ala Ile Val Phe Gly Leu Gly His Asn Gly Met
355 360 365

Ala Thr Tyr Asn Ala Asp Ala Arg Pro Asp Phe Trp Lys Leu Gln Val
370 375 380

Thr Thr Thr Arg Asn Val Thr Gly Gly His Gly Phe Pro Gln Ala Phe
385 390 395 400

Val Asp Trp Phe Cys Gly Gly Leu Gln Tyr Gln Val Asp His His Leu
405 410 415

Phe Pro Ser Leu Pro Arg His Asn Leu Ala Lys Thr His Ala Leu Val
420 425 430

Glu Ser Phe Cys Lys Glu Trp Gly Val Gln Tyr His Glu Ala Asp Leu
435 440 445

Val Asp Gly Thr Met Glu Val Leu His His Leu Gly Ser Val Ala Gly
450 455 460

Glu Phe Val Val Asp Phe Val Arg Asp Gly Pro Ala Met
465 470 475

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<400> 15

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Met Val Ser Gln Gly Gly Gly Leu Ser Gln Gly Ser Ile Glu Glu Asn
 1               5                  10                  15

att gac gtt gag cac ttg gca acg atg ccc ctc gtc agt gac ttc cta   96
Ile Asp Val Glu His Leu Ala Thr Met Pro Leu Val Ser Asp Phe Leu
            20                  25                  30

aat gtc ctg gga acg act ttg ggc cag tgg agt ctt tcc act aca ttc   144
Asn Val Leu Gly Thr Thr Leu Gly Gln Trp Ser Leu Ser Thr Thr Phe
        35                  40                  45

gct ttc aag agg ctc acg act aag aaa cac agt tcg gac atc tcg gtg   192
Ala Phe Lys Arg Leu Thr Thr Lys Lys His Ser Ser Asp Ile Ser Val
    50                  55                  60

gag gca caa aaa gaa tcg gtt gcg cgg ggg cca gtt gag aat att tct   240
Glu Ala Gln Lys Glu Ser Val Ala Arg Gly Pro Val Glu Asn Ile Ser
65                  70                  75                  80

caa tcg gtt gcg cag ccc atc agg cgg agg tgg gtg cag gat aaa aag   288
Gln Ser Val Ala Gln Pro Ile Arg Arg Arg Trp Val Gln Asp Lys Lys
                85                  90                  95

ccg gtt act tac agc ctg aag gat gta gct tcg cac gat atg ccc cag   336
Pro Val Thr Tyr Ser Leu Lys Asp Val Ala Ser His Asp Met Pro Gln
            100                 105                 110

gac tgc tgg att ata atc aaa gag aag gtg tat gat gtg agc acc ttc   384
Asp Cys Trp Ile Ile Ile Lys Glu Lys Val Tyr Asp Val Ser Thr Phe
        115                 120                 125

gct gag cag cac cct gga ggc acg gtt atc aac acc tac ttc gga cga   432
Ala Glu Gln His Pro Gly Gly Thr Val Ile Asn Thr Tyr Phe Gly Arg
    130                 135                 140

gac gcc aca gat gtt ttc tct act ttc cac gca tcc acc tca tgg aag   480
Asp Ala Thr Asp Val Phe Ser Thr Phe His Ala Ser Thr Ser Trp Lys
145                 150                 155                 160
```

```
att ctt cag aat ttc tac atc ggg aac ctt gtt agg gag gag ccg act   528
Ile Leu Gln Asn Phe Tyr Ile Gly Asn Leu Val Arg Glu Glu Pro Thr
                165                 170                 175

ttg gag ctg ctg aag gag tac aga gag ttg aga gcc ctt ttc ttg aga   576
Leu Glu Leu Leu Lys Glu Tyr Arg Glu Leu Arg Ala Leu Phe Leu Arg
            180                 185                 190

gaa cag ctt ttc aag agt tcc aaa tcc tac tac ctt ttc aag act ctc   624
Glu Gln Leu Phe Lys Ser Ser Lys Ser Tyr Tyr Leu Phe Lys Thr Leu
        195                 200                 205

ata aat gtt tcc att gtt gcc aca agc att gcg ata atc agt ctg tac   672
Ile Asn Val Ser Ile Val Ala Thr Ser Ile Ala Ile Ile Ser Leu Tyr
    210                 215                 220

aag tct tac cgg gcg gtt ctg tta tca gcc agt ttg atg ggc ttg ttt   720
Lys Ser Tyr Arg Ala Val Leu Leu Ser Ala Ser Leu Met Gly Leu Phe
225                 230                 235                 240

att caa cag tgc gga tgg ttg tct cac gat ttt cta cac cat cag gta   768
Ile Gln Gln Cys Gly Trp Leu Ser His Asp Phe Leu His His Gln Val
                245                 250                 255

ttt gag aca cgc tgg ctc aat gac gtt gtt ggc tat gtg gtc ggc aac   816
Phe Glu Thr Arg Trp Leu Asn Asp Val Val Gly Tyr Val Val Gly Asn
            260                 265                 270

gtt gtt ctg gga ttc agt gtc tcg tgg tgg aag acc aag cac aac ctg   864
Val Val Leu Gly Phe Ser Val Ser Trp Trp Lys Thr Lys His Asn Leu
        275                 280                 285

cat cat gct gct ccg aat gaa tgc gac caa aag tac aca ccg att gat   912
His His Ala Ala Pro Asn Glu Cys Asp Gln Lys Tyr Thr Pro Ile Asp
    290                 295                 300

gag gat att gat act ctc ccc atc att gct tgg agt aaa gat ctc ttg   960
Glu Asp Ile Asp Thr Leu Pro Ile Ile Ala Trp Ser Lys Asp Leu Leu
305                 310                 315                 320
```

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gcc act gtt gag agc aag acc atg ttg cga gtt ctt cag tac cag cac   1008
Ala Thr Val Glu Ser Lys Thr Met Leu Arg Val Leu Gln Tyr Gln His
                325                 330                 335

cta ttc ttt ttg gtt ctt ttg acg ttt gcc cgg gcg agt tgg cta ttt   1056
Leu Phe Phe Leu Val Leu Leu Thr Phe Ala Arg Ala Ser Trp Leu Phe
            340                 345                 350

tgg agc gcg gcc ttc act ctc agg ccc gag ttg acc ctt ggc gag aag   1104
Trp Ser Ala Ala Phe Thr Leu Arg Pro Glu Leu Thr Leu Gly Glu Lys
        355                 360                 365

ctt ttg gag agg gga acg atg gct ttg cac tac att tgg ttt aat agt   1152
Leu Leu Glu Arg Gly Thr Met Ala Leu His Tyr Ile Trp Phe Asn Ser
    370                 375                 380

gtt gcg ttt tat ctg ctc ccc gga tgg aaa cca gtt gta tgg atg gtg   1200
Val Ala Phe Tyr Leu Leu Pro Gly Trp Lys Pro Val Val Trp Met Val
385                 390                 395                 400

gtc agc gag ctc atg tct ggt ttc ctg ctg gga tac gta ttt gta ctc   1248
Val Ser Glu Leu Met Ser Gly Phe Leu Leu Gly Tyr Val Phe Val Leu
                405                 410                 415

agt cac aat gga atg gag gtg tac aat acg tca aag gac ttc gtg aat   1296
Ser His Asn Gly Met Glu Val Tyr Asn Thr Ser Lys Asp Phe Val Asn
            420                 425                 430

gcc cag att gca tcg act cgc gac atc aaa gca ggg gtg ttt aat gat   1344
Ala Gln Ile Ala Ser Thr Arg Asp Ile Lys Ala Gly Val Phe Asn Asp
        435                 440                 445

tgg ttc acc gga ggt ctc aac aga cag att gag cat cat cta ttt cca   1392
Trp Phe Thr Gly Gly Leu Asn Arg Gln Ile Glu His His Leu Phe Pro
    450                 455                 460

acg atg ccc agg cac aac ctt aat aaa att tct cct cac gtg gag act   1440
Thr Met Pro Arg His Asn Leu Asn Lys Ile Ser Pro His Val Glu Thr
465                 470                 475                 480
```

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ttg tgc aag aag cat gga ctg gtc tac gaa gac gtg agc atg gct tcg   1488
Leu Cys Lys Lys His Gly Leu Val Tyr Glu Asp Val Ser Met Ala Ser
                485                 490                 495

ggc act tac cgg gtt ttg aaa aca ctt aag gac gtt gcc gat gct gct   1536
Gly Thr Tyr Arg Val Leu Lys Thr Leu Lys Asp Val Ala Asp Ala Ala
            500                 505                 510

tca cac cag cag ctt gct gcg agt tga                              1563
Ser His Gln Gln Leu Ala Ala Ser
        515                 520
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<210> 16
<211> 520
<212> PRT
<213> Coratodon purpureus

<400> 16

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Met Val Ser Gln Gly Gly Gly Leu Ser Gln Gly Ser Ile Glu Glu Asn
  1               5                  10                  15

Ile Asp Val Glu His Leu Ala Thr Met Pro Leu Val Ser Asp Phe Leu
            20                  25                  30

Asn Val Leu Gly Thr Thr Leu Gly Gln Trp Ser Leu Ser Thr Thr Phe
        35                  40                  45

Ala Phe Lys Arg Leu Thr Thr Lys Lys His Ser Ser Asp Ile Ser Val
    50                  55                  60

Glu Ala Gln Lys Glu Ser Val Ala Arg Gly Pro Val Glu Asn Ile Ser
65                  70                  75                  80

Gln Ser Val Ala Gln Pro Ile Arg Arg Arg Trp Val Gln Asp Lys Lys
                85                  90                  95

Pro Val Thr Tyr Ser Leu Lys Asp Val Ala Ser His Asp Met Pro Gln
            100                 105                 110
```

```
Asp Cys Trp Ile Ile Ile Lys Glu Lys Val Tyr Asp Val Ser Thr Phe
        115                 120                 125

Ala Glu Gln His Pro Gly Gly Thr Val Ile Asn Thr Tyr Phe Gly Arg
    130                 135                 140

Asp Ala Thr Asp Val Phe Ser Thr Phe His Ala Ser Thr Ser Trp Lys
145                 150                 155                 160

Ile Leu Gln Asn Phe Tyr Ile Gly Asn Leu Val Arg Glu Glu Pro Thr
                165                 170                 175

Leu Glu Leu Leu Lys Glu Tyr Arg Glu Leu Arg Ala Leu Phe Leu Arg
            180                 185                 190

Glu Gln Leu Phe Lys Ser Ser Lys Ser Tyr Tyr Leu Phe Lys Thr Leu
        195                 200                 205

Ile Asn Val Ser Ile Val Ala Thr Ser Ile Ala Ile Ile Ser Leu Tyr
    210                 215                 220

Lys Ser Tyr Arg Ala Val Leu Leu Ser Ala Ser Leu Met Gly Leu Phe
225                 230                 235                 240

Ile Gln Gln Cys Gly Trp Leu Ser His Asp Phe Leu His His Gln Val
                245                 250                 255

Phe Glu Thr Arg Trp Leu Asn Asp Val Val Gly Tyr Val Val Gly Asn
            260                 265                 270

Val Val Leu Gly Phe Ser Val Ser Trp Trp Lys Thr Lys His Asn Leu
        275                 280                 285

His His Ala Ala Pro Asn Glu Cys Asp Gln Lys Tyr Thr Pro Ile Asp
    290                 295                 300

Glu Asp Ile Asp Thr Leu Pro Ile Ile Ala Trp Ser Lys Asp Leu Leu
305                 310                 315                 320
```

Ala Thr Val Glu Ser Lys Thr Met Leu Arg Val Leu Gln Tyr Gln His
325 330 335

Leu Phe Phe Leu Val Leu Leu Thr Phe Ala Arg Ala Ser Trp Leu Phe
340 345 350

Trp Ser Ala Ala Phe Thr Leu Arg Pro Glu Leu Thr Leu Gly Glu Lys
355 360 365

Leu Leu Glu Arg Gly Thr Met Ala Leu His Tyr Ile Trp Phe Asn Ser
370 375 380

Val Ala Phe Tyr Leu Leu Pro Gly Trp Lys Pro Val Val Trp Met Val
385 390 395 400

Val Ser Glu Leu Met Ser Gly Phe Leu Leu Gly Tyr Val Phe Val Leu
405 410 415

Ser His Asn Gly Met Glu Val Tyr Asn Thr Ser Lys Asp Phe Val Asn
420 425 430

Ala Gln Ile Ala Ser Thr Arg Asp Ile Lys Ala Gly Val Phe Asn Asp
435 440 445

Trp Phe Thr Gly Gly Leu Asn Arg Gln Ile Glu His His Leu Phe Pro
450 455 460

Thr Met Pro Arg His Asn Leu Asn Lys Ile Ser Pro His Val Glu Thr
465 470 475 480

Leu Cys Lys Lys His Gly Leu Val Tyr Glu Asp Val Ser Met Ala Ser
485 490 495

Gly Thr Tyr Arg Val Leu Lys Thr Leu Lys Asp Val Ala Asp Ala Ala
500 505 510

Ser His Gln Gln Leu Ala Ala Ser
515 520

<210> 17
<211> 1578
<212> DNA
<213> Physcomitrella patens

<220>
<221> CDS
<222> (1)..(1578)
<223> Δ6-desaturase

<400> 17

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atg gta ttc gcg ggc ggt gga ctt cag cag ggc tct ctc gaa gaa aac   48
Met Val Phe Ala Gly Gly Gly Leu Gln Gln Gly Ser Leu Glu Glu Asn
  1               5                  10                  15

atc gac gtc gag cac att gcc agt atg tct ctc ttc agc gac ttc ttc   96
Ile Asp Val Glu His Ile Ala Ser Met Ser Leu Phe Ser Asp Phe Phe
             20                  25                  30

agt tat gtg tct tca act gtt ggt tcg tgg agc gta cac agt ata caa   144
Ser Tyr Val Ser Ser Thr Val Gly Ser Trp Ser Val His Ser Ile Gln
         35                  40                  45

cct ttg aag cgc ctg acg agt aag aag cgt gtt tcg gaa agc gct gcc   192
Pro Leu Lys Arg Leu Thr Ser Lys Lys Arg Val Ser Glu Ser Ala Ala
     50                  55                  60

gtg caa tgt ata tca gct gaa gtt cag aga aat tcg agt acc cag gga   240
Val Gln Cys Ile Ser Ala Glu Val Gln Arg Asn Ser Ser Thr Gln Gly
 65                  70                  75                  80

act gcg gag gca ctc gca gaa tca gtc gtg aag ccc acg aga cga agg   288
Thr Ala Glu Ala Leu Ala Glu Ser Val Val Lys Pro Thr Arg Arg Arg
                 85                  90                  95

tca tct cag tgg aag aag tcg aca cac ccc cta tca gaa gta gca gta   336
Ser Ser Gln Trp Lys Lys Ser Thr His Pro Leu Ser Glu Val Ala Val
            100                 105                 110

cac aac aag cca agc gat tgc tgg att gtt gta aaa aac aag gtg tat   384
```

```
His Asn Lys Pro Ser Asp Cys Trp Ile Val Val Lys Asn Lys Val Tyr
        115                 120                 125

gat gtt tcc aat ttt gcg gac gag cat ccc gga gga tca gtt att agt   432
Asp Val Ser Asn Phe Ala Asp Glu His Pro Gly Gly Ser Val Ile Ser
    130                 135                 140

act tat ttt gga cga gac ggc aca gat gtt ttc tct agt ttt cat gca   480
Thr Tyr Phe Gly Arg Asp Gly Thr Asp Val Phe Ser Ser Phe His Ala
145                 150                 155                 160

gct tct aca tgg aaa att ctt caa gac ttt tac att ggt gac gtg gag   528
Ala Ser Thr Trp Lys Ile Leu Gln Asp Phe Tyr Ile Gly Asp Val Glu
                165                 170                 175

agg gtg gag ccg act cca gag ctg ctg aaa gat ttc cga gaa atg aga   576
Arg Val Glu Pro Thr Pro Glu Leu Leu Lys Asp Phe Arg Glu Met Arg
            180                 185                 190

gct ctt ttc ctg agg gag caa ctt ttc aaa agt tcg aaa ttg tac tat   624
Ala Leu Phe Leu Arg Glu Gln Leu Phe Lys Ser Ser Lys Leu Tyr Tyr
        195                 200                 205

gtt atg aag ctg ctc acg aat gtt gct att ttt gct gcg agc att gca   672
Val Met Lys Leu Leu Thr Asn Val Ala Ile Phe Ala Ala Ser Ile Ala
    210                 215                 220

ata ata tgt tgg agc aag act att tca gcg gtt ttg gct tca gct tgt   720
Ile Ile Cys Trp Ser Lys Thr Ile Ser Ala Val Leu Ala Ser Ala Cys
225                 230                 235                 240

atg atg gct ctg tgt ttc caa cag tgc gga tgg cta tcc cat gat ttt   768
Met Met Ala Leu Cys Phe Gln Gln Cys Gly Trp Leu Ser His Asp Phe
                245                 250                 255

ctc cac aat cag gtg ttt gag aca cgc tgg ctt aat gaa gtt gtc ggg   816
Leu His Asn Gln Val Phe Glu Thr Arg Trp Leu Asn Glu Val Val Gly
            260                 265                 270

tat gtg atc ggc aac gcc gtt ctg ggg ttt agt aca ggg tgg tgg aag   864
```

```
Tyr Val Ile Gly Asn Ala Val Leu Gly Phe Ser Thr Gly Trp Trp Lys
        275                 280                 285

gag aag cat aac ctt cat cat gct gct cca aat gaa tgc gat cag act   912
Glu Lys His Asn Leu His His Ala Ala Pro Asn Glu Cys Asp Gln Thr
    290                 295                 300

tac caa cca att gat gaa gat att gat act ctc ccc ctc att gcc tgg   960
Tyr Gln Pro Ile Asp Glu Asp Ile Asp Thr Leu Pro Leu Ile Ala Trp
305                 310                 315                 320

agc aag gac ata ctg gcc aca gtt gag aat aag aca ttc ttg cga atc   1008
Ser Lys Asp Ile Leu Ala Thr Val Glu Asn Lys Thr Phe Leu Arg Ile
                325                 330                 335

ctc caa tac cag cat ctg ttc ttc atg ggt ctg tta ttt ttc gcc cgt   1056
Leu Gln Tyr Gln His Leu Phe Phe Met Gly Leu Leu Phe Phe Ala Arg
            340                 345                 350

ggt agt tgg ctc ttt tgg agc tgg aga tat acc tct aca gca gtg ctc   1104
Gly Ser Trp Leu Phe Trp Ser Trp Arg Tyr Thr Ser Thr Ala Val Leu
        355                 360                 365

tca cct gtc gac agg ttg ttg gag aag gga act gtt ctg ttt cac tac   1152
Ser Pro Val Asp Arg Leu Leu Glu Lys Gly Thr Val Leu Phe His Tyr
    370                 375                 380

ttt tgg ttc gtc ggg aca gcg tgc tat ctt ctc cct ggt tgg aag cca   1200
Phe Trp Phe Val Gly Thr Ala Cys Tyr Leu Leu Pro Gly Trp Lys Pro
385                 390                 395                 400

tta gta tgg atg gcg gtg act gag ctc atg tcc ggc atg ctg ctg ggc   1248
Leu Val Trp Met Ala Val Thr Glu Leu Met Ser Gly Met Leu Leu Gly
                405                 410                 415

ttt gta ttt gta ctt agc cac aat ggg atg gag gtt tat aat tcg tct   1296
Phe Val Phe Val Leu Ser His Asn Gly Met Glu Val Tyr Asn Ser Ser
            420                 425                 430

aaa gaa ttc gtg agt gca cag atc gta tcc aca cgg gat atc aaa gga   1344
```

```
Lys Glu Phe Val Ser Ala Gln Ile Val Ser Thr Arg Asp Ile Lys Gly
        435                 440                 445

aac ata ttc aac gac tgg ttc act ggt ggc ctt aac agg caa ata gag   1392
Asn Ile Phe Asn Asp Trp Phe Thr Gly Gly Leu Asn Arg Gln Ile Glu
    450                 455                 460

cat cat ctt ttc cca aca atg ccc agg cat aat tta aac aaa ata gca   1440
His His Leu Phe Pro Thr Met Pro Arg His Asn Leu Asn Lys Ile Ala
465                 470                 475                 480

cct aga gtg gag gtg ttc tgt aag aaa cac ggt ctg gtg tac gaa gac   1488
Pro Arg Val Glu Val Phe Cys Lys Lys His Gly Leu Val Tyr Glu Asp
                485                 490                 495

gta tct att gct acc ggc act tgc aag gtt ttg aaa gca ttg aag gaa   1536
Val Ser Ile Ala Thr Gly Thr Cys Lys Val Leu Lys Ala Leu Lys Glu
            500                 505                 510

gtc gcg gag gct gcg gca gag cag cat gct acc acc agt taa           1578
Val Ala Glu Ala Ala Ala Glu Gln His Ala Thr Thr Ser
        515                 520                 525
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<210> 18
<211> 525
<212> PRT
<213> Physcomitrella patens

<400> 18

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Met Val Phe Ala Gly Gly Gly Leu Gln Gln Gly Ser Leu Glu Glu Asn
  1               5                  10                  15

Ile Asp Val Glu His Ile Ala Ser Met Ser Leu Phe Ser Asp Phe Phe
            20                  25                  30

Ser Tyr Val Ser Ser Thr Val Gly Ser Trp Ser Val His Ser Ile Gln
        35                  40                  45

Pro Leu Lys Arg Leu Thr Ser Lys Lys Arg Val Ser Glu Ser Ala Ala
```

```
     50                  55                  60

Val Gln Cys Ile Ser Ala Glu Val Gln Arg Asn Ser Ser Thr Gln Gly
 65                  70                  75                  80

Thr Ala Glu Ala Leu Ala Glu Ser Val Val Lys Pro Thr Arg Arg Arg
                 85                  90                  95

Ser Ser Gln Trp Lys Lys Ser Thr His Pro Leu Ser Glu Val Ala Val
            100                 105                 110

His Asn Lys Pro Ser Asp Cys Trp Ile Val Val Lys Asn Lys Val Tyr
        115                 120                 125

Asp Val Ser Asn Phe Ala Asp Glu His Pro Gly Gly Ser Val Ile Ser
    130                 135                 140

Thr Tyr Phe Gly Arg Asp Gly Thr Asp Val Phe Ser Ser Phe His Ala
145                 150                 155                 160

Ala Ser Thr Trp Lys Ile Leu Gln Asp Phe Tyr Ile Gly Asp Val Glu
                165                 170                 175

Arg Val Glu Pro Thr Pro Glu Leu Leu Lys Asp Phe Arg Glu Met Arg
            180                 185                 190

Ala Leu Phe Leu Arg Glu Gln Leu Phe Lys Ser Ser Lys Leu Tyr Tyr
        195                 200                 205

Val Met Lys Leu Leu Thr Asn Val Ala Ile Phe Ala Ala Ser Ile Ala
    210                 215                 220

Ile Ile Cys Trp Ser Lys Thr Ile Ser Ala Val Leu Ala Ser Ala Cys
225                 230                 235                 240

Met Met Ala Leu Cys Phe Gln Gln Cys Gly Trp Leu Ser His Asp Phe
                245                 250                 255

Leu His Asn Gln Val Phe Glu Thr Arg Trp Leu Asn Glu Val Val Gly
            260                 265                 270
```

Tyr Val Ile Gly Asn Ala Val Leu Gly Phe Ser Thr Gly Trp Trp Lys
275 280 285

Glu Lys His Asn Leu His His Ala Ala Pro Asn Glu Cys Asp Gln Thr
290 295 300

Tyr Gln Pro Ile Asp Glu Asp Ile Asp Thr Leu Pro Leu Ile Ala Trp
305 310 315 320

Ser Lys Asp Ile Leu Ala Thr Val Glu Asn Lys Thr Phe Leu Arg Ile
325 330 335

Leu Gln Tyr Gln His Leu Phe Phe Met Gly Leu Leu Phe Phe Ala Arg
340 345 350

Gly Ser Trp Leu Phe Trp Ser Trp Arg Tyr Thr Ser Thr Ala Val Leu
355 360 365

Ser Pro Val Asp Arg Leu Leu Glu Lys Gly Thr Val Leu Phe His Tyr
370 375 380

Phe Trp Phe Val Gly Thr Ala Cys Tyr Leu Leu Pro Gly Trp Lys Pro
385 390 395 400

Leu Val Trp Met Ala Val Thr Glu Leu Met Ser Gly Met Leu Leu Gly
405 410 415

Phe Val Phe Val Leu Ser His Asn Gly Met Glu Val Tyr Asn Ser Ser
420 425 430

Lys Glu Phe Val Ser Ala Gln Ile Val Ser Thr Arg Asp Ile Lys Gly
435 440 445

Asn Ile Phe Asn Asp Trp Phe Thr Gly Gly Leu Asn Arg Gln Ile Glu
450 455 460

His His Leu Phe Pro Thr Met Pro Arg His Asn Leu Asn Lys Ile Ala
465 470 475 480

Pro Arg Val Glu Val Phe Cys Lys Lys His Gly Leu Val Tyr Glu Asp
485 490 495

Val Ser Ile Ala Thr Gly Thr Cys Lys Val Leu Lys Ala Leu Lys Glu
500 505 510

Val Ala Glu Ala Ala Ala Glu Gln His Ala Thr Thr Ser
515 520 525

<210> 19
<211> 837
<212> DNA
<213> Phytophthora infestans

<220>
<221> CDS
<222> (1)..(837)
<223> Δ6-elongase

<400> 19
atg tcg act gag cta ctg cag agc tac tac gcg tgg gcc aac gcc acg 48
Met Ser Thr Glu Leu Leu Gln Ser Tyr Tyr Ala Trp Ala Asn Ala Thr
1 5 10 15

gag gcc aag ctg ctg gac tgg gtc gac cct gag ggc ggc tgg aag gtg 96
Glu Ala Lys Leu Leu Asp Trp Val Asp Pro Glu Gly Gly Trp Lys Val
20 25 30

cat cct atg gca gac tac ccc cta gcc aac ttc tcc agc gtc tac gcc 144
His Pro Met Ala Asp Tyr Pro Leu Ala Asn Phe Ser Ser Val Tyr Ala
35 40 45

atc tgc gtc gga tac ttg ctc ttc gta atc ttc ggc acg gcc ctg atg 192
Ile Cys Val Gly Tyr Leu Leu Phe Val Ile Phe Gly Thr Ala Leu Met
50 55 60

aaa atg gga gtc ccc gcc atc aag acc agt cca tta cag ttt gtg tac 240
Lys Met Gly Val Pro Ala Ile Lys Thr Ser Pro Leu Gln Phe Val Tyr
65 70 75 80

```
aac ccc atc caa gtc att gcc tgc tct tat atg tgc gtg gag gcc gcc   288
Asn Pro Ile Gln Val Ile Ala Cys Ser Tyr Met Cys Val Glu Ala Ala
                85                  90                  95

atc cag gcc tac cgc aac ggc tac acc gcc gcc ccg tgc aac gcc ttt   336
Ile Gln Ala Tyr Arg Asn Gly Tyr Thr Ala Ala Pro Cys Asn Ala Phe
            100                 105                 110

aag tcc gac gac ccc gtc atg ggc aac gtt ctg tac ctc ttc tat ctc   384
Lys Ser Asp Asp Pro Val Met Gly Asn Val Leu Tyr Leu Phe Tyr Leu
        115                 120                 125

tcc aag atg ctc gac ctg tgc gac aca gtc ttc att atc cta gga aag   432
Ser Lys Met Leu Asp Leu Cys Asp Thr Val Phe Ile Ile Leu Gly Lys
    130                 135                 140

aag tgg aaa cag ctt tcc atc ttg cac gtg tac cac cac ctt acc gtg   480
Lys Trp Lys Gln Leu Ser Ile Leu His Val Tyr His His Leu Thr Val
145                 150                 155                 160

ctt ttc gtc tac tat gtg acg ttc cgc gcc gct cag gac ggg gac tca   528
Leu Phe Val Tyr Tyr Val Thr Phe Arg Ala Ala Gln Asp Gly Asp Ser
                165                 170                 175

tat gct acc atc gtg ctc aac ggc ttc gtg cac acc atc atg tac act   576
Tyr Ala Thr Ile Val Leu Asn Gly Phe Val His Thr Ile Met Tyr Thr
            180                 185                 190

tac tac ttc gtc agc gcc cac acg cgc aac att tgg tgg aag aag tac   624
Tyr Tyr Phe Val Ser Ala His Thr Arg Asn Ile Trp Trp Lys Lys Tyr
        195                 200                 205

ctc acg cgc att cag ctt atc cag ttc gtg acc atg aac gtg cag ggc   672
Leu Thr Arg Ile Gln Leu Ile Gln Phe Val Thr Met Asn Val Gln Gly
    210                 215                 220

tac ctg acc tac tct cga cag tgc cca ggc atg cct cct aag gtg ccg   720
Tyr Leu Thr Tyr Ser Arg Gln Cys Pro Gly Met Pro Pro Lys Val Pro
225                 230                 235                 240
```

```
ctc atg tac ctt gtg tac gtg cag tca ctc ttc tgg ctc ttc atg aat   768
Leu Met Tyr Leu Val Tyr Val Gln Ser Leu Phe Trp Leu Phe Met Asn
                245                 250                 255

ttc tac att cgc gcg tac gtg ttc ggc ccc aag aaa ccg gcc gtg gag   816
Phe Tyr Ile Arg Ala Tyr Val Phe Gly Pro Lys Lys Pro Ala Val Glu
            260                 265                 270

gaa tcg aag aag aag ttg taa                                      837
Glu Ser Lys Lys Lys Leu
        275
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<210> 20
<211> 278
<212> PRT
<213> Phytophthora infestans

<400> 20

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Met Ser Thr Glu Leu Leu Gln Ser Tyr Tyr Ala Trp Ala Asn Ala Thr
  1               5                  10                  15

Glu Ala Lys Leu Leu Asp Trp Val Asp Pro Glu Gly Gly Trp Lys Val
            20                  25                  30

His Pro Met Ala Asp Tyr Pro Leu Ala Asn Phe Ser Ser Val Tyr Ala
        35                  40                  45

Ile Cys Val Gly Tyr Leu Leu Phe Val Ile Phe Gly Thr Ala Leu Met
    50                  55                  60

Lys Met Gly Val Pro Ala Ile Lys Thr Ser Pro Leu Gln Phe Val Tyr
65                  70                  75                  80

Asn Pro Ile Gln Val Ile Ala Cys Ser Tyr Met Cys Val Glu Ala Ala
                85                  90                  95

Ile Gln Ala Tyr Arg Asn Gly Tyr Thr Ala Ala Pro Cys Asn Ala Phe
            100                 105                 110
```

Lys Ser Asp Asp Pro Val Met Gly Asn Val Leu Tyr Leu Phe Tyr Leu
115 120 125

Ser Lys Met Leu Asp Leu Cys Asp Thr Val Phe Ile Ile Leu Gly Lys
130 135 140

Lys Trp Lys Gln Leu Ser Ile Leu His Val Tyr His His Leu Thr Val
145 150 155 160

Leu Phe Val Tyr Tyr Val Thr Phe Arg Ala Ala Gln Asp Gly Asp Ser
165 170 175

Tyr Ala Thr Ile Val Leu Asn Gly Phe Val His Thr Ile Met Tyr Thr
180 185 190

Tyr Tyr Phe Val Ser Ala His Thr Arg Asn Ile Trp Trp Lys Lys Tyr
195 200 205

Leu Thr Arg Ile Gln Leu Ile Gln Phe Val Thr Met Asn Val Gln Gly
210 215 220

Tyr Leu Thr Tyr Ser Arg Gln Cys Pro Gly Met Pro Pro Lys Val Pro
225 230 235 240

Leu Met Tyr Leu Val Tyr Val Gln Ser Leu Phe Trp Leu Phe Met Asn
245 250 255

Phe Tyr Ile Arg Ala Tyr Val Phe Gly Pro Lys Lys Pro Ala Val Glu
260 265 270

Glu Ser Lys Lys Lys Leu
275

<210> 21
<211> 1410
<212> DNA
<213> Phaeodactylum tricornutum

<220>
<221> CDS
<222> (1)..(1410)
<223> Δ5-desaturase

<400> 21

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atg gct ccg gat gcg gat aag ctt cga caa cgc cag acg act gcg gta   48
Met Ala Pro Asp Ala Asp Lys Leu Arg Gln Arg Gln Thr Thr Ala Val
 1               5                  10                  15

gcg aag cac aat gct gct acc ata tcg acg cag gaa cgc ctt tgc agt   96
Ala Lys His Asn Ala Ala Thr Ile Ser Thr Gln Glu Arg Leu Cys Ser
            20                  25                  30

ctg tct tcg ctc aaa ggc gaa gaa gtc tgc atc gac gga atc atc tat  144
Leu Ser Ser Leu Lys Gly Glu Glu Val Cys Ile Asp Gly Ile Ile Tyr
        35                  40                  45

gac ctc caa tca ttc gat cat ccc ggg ggt gaa acg atc aaa atg ttt  192
Asp Leu Gln Ser Phe Asp His Pro Gly Gly Glu Thr Ile Lys Met Phe
    50                  55                  60

ggt ggc aac gat gtc act gta cag tac aag atg att cac ccg tac cat  240
Gly Gly Asn Asp Val Thr Val Gln Tyr Lys Met Ile His Pro Tyr His
65                  70                  75                  80

acc gag aag cat ttg gaa aag atg aag cgt gtc ggc aag gtg acg gat  288
Thr Glu Lys His Leu Glu Lys Met Lys Arg Val Gly Lys Val Thr Asp
                85                  90                  95

ttc gtc tgc gag tac aag ttc gat acc gaa ttt gaa cgc gaa atc aaa  336
Phe Val Cys Glu Tyr Lys Phe Asp Thr Glu Phe Glu Arg Glu Ile Lys
            100                 105                 110

cga gaa gtc ttc aag att gtg cga cga ggc aag gat ttc ggt act ttg  384
Arg Glu Val Phe Lys Ile Val Arg Arg Gly Lys Asp Phe Gly Thr Leu
        115                 120                 125

gga tgg ttc ttc cgt gcg ttt tgc tac att gcc att ttc ttc tac ctg  432
Gly Trp Phe Phe Arg Ala Phe Cys Tyr Ile Ala Ile Phe Phe Tyr Leu
```

```
130                 135                 140

cag tac cat tgg gtc acc acg gga acc tct tgg ctg ctg gcc gtg gcc   480
Gln Tyr His Trp Val Thr Thr Gly Thr Ser Trp Leu Leu Ala Val Ala
145                 150                 155                 160

tac gga atc tcc caa gcg atg att ggc atg aat gtc cag cac gat gcc   528
Tyr Gly Ile Ser Gln Ala Met Ile Gly Met Asn Val Gln His Asp Ala
                165                 170                 175

aac cac ggg gcc acc tcc aag cgt ccc tgg gtc aac gac atg cta ggc   576
Asn His Gly Ala Thr Ser Lys Arg Pro Trp Val Asn Asp Met Leu Gly
            180                 185                 190

ctc ggt gcg gat ttt att ggt ggt tcc aag tgg ctc tgg cag gaa caa   624
Leu Gly Ala Asp Phe Ile Gly Gly Ser Lys Trp Leu Trp Gln Glu Gln
        195                 200                 205

cac tgg acc cac cac gct tac acc aat cac gcc gag atg gat ccc gat   672
His Trp Thr His His Ala Tyr Thr Asn His Ala Glu Met Asp Pro Asp
    210                 215                 220

agc ttt ggt gcc gaa cca atg ctc cta ttc aac gac tat ccc ttg gat   720
Ser Phe Gly Ala Glu Pro Met Leu Leu Phe Asn Asp Tyr Pro Leu Asp
225                 230                 235                 240

cat ccc gct cgt acc tgg cta cat cgc ttt caa gca ttc ttt tac atg   768
His Pro Ala Arg Thr Trp Leu His Arg Phe Gln Ala Phe Phe Tyr Met
                245                 250                 255

ccc gtc ttg gct gga tac tgg ttg tcc gct gtc ttc aat cca caa att   816
Pro Val Leu Ala Gly Tyr Trp Leu Ser Ala Val Phe Asn Pro Gln Ile
            260                 265                 270

ctt gac ctc cag caa cgc ggc gca ctt tcc gtc ggt atc cgt ctc gac   864
Leu Asp Leu Gln Gln Arg Gly Ala Leu Ser Val Gly Ile Arg Leu Asp
        275                 280                 285

aac gct ttc att cac tcg cga cgc aag tat gcg gtt ttc tgg cgg gct   912
Asn Ala Phe Ile His Ser Arg Arg Lys Tyr Ala Val Phe Trp Arg Ala
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290                 295                 300

gtg tac att gcg gtg aac gtg att gct ccg ttt tac aca aac tcc ggc   960
Val Tyr Ile Ala Val Asn Val Ile Ala Pro Phe Tyr Thr Asn Ser Gly
305                 310                 315                 320

ctc gaa tgg tcc tgg cgt gtc ttt gga aac atc atg ctc atg ggt gtg   1008
Leu Glu Trp Ser Trp Arg Val Phe Gly Asn Ile Met Leu Met Gly Val
                325                 330                 335

gcg gaa tcg ctc gcg ctg gcg gtc ctg ttt tcg ttg tcg cac aat ttc   1056
Ala Glu Ser Leu Ala Leu Ala Val Leu Phe Ser Leu Ser His Asn Phe
            340                 345                 350

gaa tcc gcg gat cgc gat ccg acc gcc cca ctg aaa aag acg gga gaa   1104
Glu Ser Ala Asp Arg Asp Pro Thr Ala Pro Leu Lys Lys Thr Gly Glu
        355                 360                 365

cca gtc gac tgg ttc aag aca cag gtc gaa act tcc tgc act tac ggt   1152
Pro Val Asp Trp Phe Lys Thr Gln Val Glu Thr Ser Cys Thr Tyr Gly
    370                 375                 380

gga ttc ctt tcc ggt tgc ttc acg gga ggt ctc aac ttt cag gtt gaa   1200
Gly Phe Leu Ser Gly Cys Phe Thr Gly Gly Leu Asn Phe Gln Val Glu
385                 390                 395                 400

cac cac ttg ttc cca cgc atg agc agc gct tgg tat ccc tac att gcc   1248
His His Leu Phe Pro Arg Met Ser Ser Ala Trp Tyr Pro Tyr Ile Ala
                405                 410                 415

ccc aag gtc cgc gaa att tgc gcc aaa cac ggc gtc cac tac gcc tac   1296
Pro Lys Val Arg Glu Ile Cys Ala Lys His Gly Val His Tyr Ala Tyr
            420                 425                 430

tac ccg tgg atc cac caa aac ttt ctc tcc acc gtc cgc tac atg cac   1344
Tyr Pro Trp Ile His Gln Asn Phe Leu Ser Thr Val Arg Tyr Met His
        435                 440                 445

gcg gcc ggg acc ggt gcc aac tgg cgc cag atg gcc aga gaa aat ccc   1392
Ala Ala Gly Thr Gly Ala Asn Trp Arg Gln Met Ala Arg Glu Asn Pro
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450 455 460

ttg acc gga cgg gcg taa 1410
Leu Thr Gly Arg Ala
465 470

<210> 22
<211> 469
<212> PRT
<213> Phaeodactylum tricornutum

<400> 22
Met Ala Pro Asp Ala Asp Lys Leu Arg Gln Arg Gln Thr Thr Ala Val
1 5 10 15

Ala Lys His Asn Ala Ala Thr Ile Ser Thr Gln Glu Arg Leu Cys Ser
20 25 30

Leu Ser Ser Leu Lys Gly Glu Glu Val Cys Ile Asp Gly Ile Ile Tyr
35 40 45

Asp Leu Gln Ser Phe Asp His Pro Gly Gly Glu Thr Ile Lys Met Phe
50 55 60

Gly Gly Asn Asp Val Thr Val Gln Tyr Lys Met Ile His Pro Tyr His
65 70 75 80

Thr Glu Lys His Leu Glu Lys Met Lys Arg Val Gly Lys Val Thr Asp
85 90 95

Phe Val Cys Gln Tyr Lys Phe Asp Thr Glu Phe Glu Arg Glu Ile Lys
100 105 110

Arg Glu Val Phe Lys Ile Val Arg Arg Gly Lys Asp Phe Gly Thr Leu
115 120 125

Gly Trp Phe Phe Arg Ala Phe Cys Tyr Ile Ala Ile Phe Phe Tyr Leu
130 135 140

Gln Tyr His Trp Val Thr Thr Gly Thr Ser Trp Leu Leu Ala Val Ala
145 150 155 160

Tyr Gly Ile Ser Gln Ala Met Ile Gly Met Asn Val Glu His Asp Ala
165 170 175

Asn His Gly Ala Thr Ser Lys Arg Pro Trp Val Asn Asp Met Leu Gly
180 185 190

Leu Gly Ala Asp Phe Ile Gly Gly Ser Lys Trp Leu Trp Gln Glu Gln
195 200 205

His Trp Thr His His Ala Tyr Thr Asn His Ala Glu Met Asp Pro Asp
210 215 220

Ser Phe Gly Ala Glu Pro Met Leu Leu Phe Asn Asp Tyr Pro Leu Asp
225 230 235 240

His Pro Ala Arg Thr Trp Leu His Arg Phe Gln Ala Phe Phe Tyr Met
245 250 255

Pro Val Leu Ala Gly Tyr Trp Leu Ser Ala Val Phe Asn Pro Gln Ile
260 265 270

Leu Asp Leu Gln Gln Arg Gly Ala Leu Ser Val Gly Ile Arg Leu Asp
275 280 285

Asn Ala Phe Ile His Ser Arg Arg Lys Tyr Ala Val Phe Trp Arg Ala
290 295 300

Val Tyr Ile Ala Val Asn Val Ile Ala Pro Phe Tyr Thr Asn Ser Gly
305 310 315 320

Leu Glu Trp Ser Trp Arg Val Phe Gly Asn Ile Met Leu Met Gly Val
325 330 335

Ala Glu Ser Leu Ala Leu Ala Val Leu Phe Ser Leu Ser His Asn Phe
340 345 350

Glu Ser Ala Asp Arg Asp Pro Thr Ala Pro Leu Lys Lys Thr Gly Glu

355 360 365

Pro Val Asp Trp Phe Lys Thr Gln Val Glu Thr Ser Cys Thr Tyr Gly
370 375 380

Gly Phe Leu Ser Gly Cys Phe Thr Gly Gly Leu Asn Phe Gln Val Glu
385 390 395 400

His His Leu Phe Pro Arg Met Ser Ser Ala Trp Tyr Pro Tyr Ile Ala
405 410 415

Pro Lys Val Arg Glu Ile Cys Ala Lys His Gly Val His Tyr Ala Tyr
420 425 430

Tyr Pro Trp Ile His Gln Asn Phe Leu Ser Thr Val Arg Tyr Met His
435 440 445

Ala Ala Gly Thr Gly Ala Asn Trp Arg Gln Met Ala Arg Glu Asn Pro
450 455 460

Leu Thr Gly Arg Ala
465

<210> 23
<211> 1344
<212> DNA
<213> Caenorhabditis elegans

<220>
<221> CDS
<222> (1)..(1344)
<223> Δ5-desaturase

<400> 23
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Met Val Leu Arg Glu Gln Glu His Glu Pro Phe Phe Ile Lys Ile Asp
1 5 10 15

gga aaa tgg tgt caa att gac gat gct gtc ctg aga tca cat cca ggt 96

```
Gly Lys Trp Cys Gln Ile Asp Asp Ala Val Leu Arg Ser His Pro Gly
            20                  25                  30

ggt agt gca att act acc tat aaa aat atg gat gcc act acc gta ttc   144
Gly Ser Ala Ile Thr Thr Tyr Lys Asn Met Asp Ala Thr Thr Val Phe
        35                  40                  45

cac aca ttc cat act ggt tct aaa gaa gcg tat caa tgg ctg aca gaa   192
His Thr Phe His Thr Gly Ser Lys Glu Ala Tyr Gln Trp Leu Thr Glu
    50                  55                  60

ttg aaa aaa gag tgc cct aca caa gaa cca gag atc cca gat att aag   240
Leu Lys Lys Glu Cys Pro Thr Gln Glu Pro Glu Ile Pro Asp Ile Lys
65                  70                  75                  80

gat gac cca atc aaa gga att gat gat gtg aac atg gga act ttc aat   288
Asp Asp Pro Ile Lys Gly Ile Asp Asp Val Asn Met Gly Thr Phe Asn
                85                  90                  95

att tct gag aaa cga tct gcc caa ata aat aaa agt ttc act gat cta   336
Ile Ser Glu Lys Arg Ser Ala Gln Ile Asn Lys Ser Phe Thr Asp Leu
            100                 105                 110

cgt atg cga gtt cgt gca gaa gga ctt atg gat gga tct cct ttg ttc   384
Arg Met Arg Val Arg Ala Glu Gly Leu Met Asp Gly Ser Pro Leu Phe
        115                 120                 125

tac att aga aaa att ctt gaa aca atc ttc aca att ctt ttt gca ttc   432
Tyr Ile Arg Lys Ile Leu Glu Thr Ile Phe Thr Ile Leu Phe Ala Phe
    130                 135                 140

tac ctt caa tac cac aca tat tat ctt cca tca gct att cta atg gga   480
Tyr Leu Gln Tyr His Thr Tyr Tyr Leu Pro Ser Ala Ile Leu Met Gly
145                 150                 155                 160

gtt gcg tgg caa caa ttg gga tgg tta atc cat gaa ttc gca cat cat   528
Val Ala Trp Gln Gln Leu Gly Trp Leu Ile His Glu Phe Ala His His
                165                 170                 175

cag ttg ttc aaa aac aga tac tac aat gat ttg gcc agc tat ttc gtt   576
```

```
Gln Leu Phe Lys Asn Arg Tyr Tyr Asn Asp Leu Ala Ser Tyr Phe Val
            180                 185                 190

gga aac ttt tta caa gga ttc tca tct ggt ggt tgg aaa gag cag cac   624
Gly Asn Phe Leu Gln Gly Phe Ser Ser Gly Gly Trp Lys Glu Gln His
        195                 200                 205

aat gtg cat cac gca gcc aca aat gtt gtt gga cga gac gga gat ctt   672
Asn Val His His Ala Ala Thr Asn Val Val Gly Arg Asp Gly Asp Leu
    210                 215                 220

gat tta gtc cca ttc tat gct aca gtg gca gaa cat ctc aac aat tat   720
Asp Leu Val Pro Phe Tyr Ala Thr Val Ala Glu His Leu Asn Asn Tyr
225                 230                 235                 240

tct cag gat tca tgg gtt atg act cta ttc aga tgg caa cat gtt cat   768
Ser Gln Asp Ser Trp Val Met Thr Leu Phe Arg Trp Gln His Val His
                245                 250                 255

tgg aca ttc atg tta cca ttc ctc cgt ctc tcg tgg ctt ctt cag tca   816
Trp Thr Phe Met Leu Pro Phe Leu Arg Leu Ser Trp Leu Leu Gln Ser
            260                 265                 270

atc att ttt gtt agt cag atg cca act cat tat tat gac tat tac aga   864
Ile Ile Phe Val Ser Gln Met Pro Thr His Tyr Tyr Asp Tyr Tyr Arg
        275                 280                 285

aat act gcg att tat gaa cag gtt ggt ctc tct ttg cac tgg gct tgg   912
Asn Thr Ala Ile Tyr Glu Gln Val Gly Leu Ser Leu His Trp Ala Trp
    290                 295                 300

tca ttg ggt caa ttg tat ttc cta ccc gat tgg tca act aga ata atg   960
Ser Leu Gly Gln Leu Tyr Phe Leu Pro Asp Trp Ser Thr Arg Ile Met
305                 310                 315                 320

ttc ttc ctt gtt tct cat ctt gtt gga ggt ttc ctg ctc tct cat gta  1008
Phe Phe Leu Val Ser His Leu Val Gly Gly Phe Leu Leu Ser His Val
                325                 330                 335

gtt act ttc aat cat tat tca gtg gag aag ttt gca ttg agc tcg aac  1056
```

```
Val Thr Phe Asn His Tyr Ser Val Glu Lys Phe Ala Leu Ser Ser Asn
            340                 345                 350

atc atg tca aat tac gct tgt ctt caa atc atg acc aca aga aat atg   1104
Ile Met Ser Asn Tyr Ala Cys Leu Gln Ile Met Thr Thr Arg Asn Met
        355                 360                 365

aga cct gga aga ttc att gac tgg ctt tgg gga ggt ctt aac tat cag   1152
Arg Pro Gly Arg Phe Ile Asp Trp Leu Trp Gly Gly Leu Asn Tyr Gln
    370                 375                 380

att gag cac cat ctt ttc cca acg atg cca cga cac aac ttg aac act   1200
Ile Glu His His Leu Phe Pro Thr Met Pro Arg His Asn Leu Asn Thr
385                 390                 395                 400

gtt atg cca ctt gtt aag gag ttt gca gca gca aat ggt tta cca tac   1248
Val Met Pro Leu Val Lys Glu Phe Ala Ala Ala Asn Gly Leu Pro Tyr
                405                 410                 415

atg gtc gac gat tat ttc aca gga ttc tgg ctt gaa att gag caa ttc   1296
Met Val Asp Asp Tyr Phe Thr Gly Phe Trp Leu Glu Ile Glu Gln Phe
            420                 425                 430

cga aat att gca aat gtt gct gct aaa ttg act aaa aag att gcc tag   1344
Arg Asn Ile Ala Asn Val Ala Ala Lys Leu Thr Lys Lys Ile Ala
        435                 440                 445
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<210> 24
<211> 447
<212> PRT
<213> Caenorhabditis elegans

<400> 24

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Met Val Leu Arg Glu Gln Glu His Glu Pro Phe Phe Ile Lys Ile Asp
  1               5                  10                  15

Gly Lys Trp Cys Gln Ile Asp Asp Ala Val Leu Arg Ser His Pro Gly
            20                  25                  30
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Gly Ser Ala Ile Thr Thr Tyr Lys Asn Met Asp Ala Thr Thr Val Phe
35 40 45

His Thr Phe His Thr Gly Ser Lys Glu Ala Tyr Gln Trp Leu Thr Glu
50 55 60

Leu Lys Lys Glu Cys Pro Thr Gln Glu Pro Glu Ile Pro Asp Ile Lys
65 70 75 80

Asp Asp Pro Ile Lys Gly Ile Asp Asp Val Asn Met Gly Thr Phe Asn
85 90 95

Ile Ser Glu Lys Arg Ser Ala Gln Ile Asn Lys Ser Phe Thr Asp Leu
100 105 110

Arg Met Arg Val Arg Ala Glu Gly Leu Met Asp Gly Ser Pro Leu Phe
115 120 125

Tyr Ile Arg Lys Ile Leu Glu Thr Ile Phe Thr Ile Leu Phe Ala Phe
130 135 140

Tyr Leu Gln Tyr His Thr Tyr Tyr Leu Pro Ser Ala Ile Leu Met Gly
145 150 155 160

Val Ala Trp Gln Gln Leu Gly Trp Leu Ile His Glu Phe Ala His His
165 170 175

Gln Leu Phe Lys Asn Arg Tyr Tyr Asn Asp Leu Ala Ser Tyr Phe Val
180 185 190

Gly Asn Phe Leu Gln Gly Phe Ser Ser Gly Gly Trp Lys Glu Gln His
195 200 205

Asn Val His His Ala Ala Thr Asn Val Val Gly Arg Asp Gly Asp Leu
210 215 220

Asp Leu Val Pro Phe Tyr Ala Thr Val Ala Glu His Leu Asn Asn Tyr
225 230 235 240

Ser Gln Asp Ser Trp Val Met Thr Leu Phe Arg Trp Gln His Val His

245 250 255

Trp Thr Phe Met Leu Pro Phe Leu Arg Leu Ser Trp Leu Leu Gln Ser
260 265 270

Ile Ile Phe Val Ser Gln Met Pro Thr His Tyr Tyr Asp Tyr Tyr Arg
275 280 285

Asn Thr Ala Ile Tyr Glu Gln Val Gly Leu Ser Leu His Trp Ala Trp
290 295 300

Ser Leu Gly Gln Leu Tyr Phe Leu Pro Asp Trp Ser Thr Arg Ile Met
305 310 315 320

Phe Phe Leu Val Ser His Leu Val Gly Gly Phe Leu Leu Ser His Val
325 330 335

Val Thr Phe Asn His Tyr Ser Val Glu Lys Phe Ala Leu Ser Ser Asn
340 345 350

Ile Met Ser Asn Tyr Ala Cys Leu Gln Ile Met Thr Thr Arg Asn Met
355 360 365

Arg Pro Gly Arg Phe Ile Asp Trp Leu Trp Gly Gly Leu Asn Tyr Gln
370 375 380

Ile Glu His His Leu Phe Pro Thr Met Pro Arg His Asn Leu Asn Thr
385 390 395 400

Val Met Pro Leu Val Lys Glu Phe Ala Ala Ala Asn Gly Leu Pro Tyr
405 410 415

Met Val Asp Asp Tyr Phe Thr Gly Phe Trp Leu Glu Ile Glu Gln Phe
420 425 430

Arg Asn Ile Ala Asn Val Ala Ala Lys Leu Thr Lys Lys Ile Ala
435 440 445

<210> 25

<211> 954
<212> DNA
<213> Mortierella alpina

<220>
<221> CDS
<222> (1)..(954)
<223> Δ6-elongase

<400> 25

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atg gcc gcc gca atc ttg gac aag gtc aac ttc ggc att gat cag ccc   48
Met Ala Ala Ala Ile Leu Asp Lys Val Asn Phe Gly Ile Asp Gln Pro
 1               5                  10                  15

ttc gga atc aag ctc gac acc tac ttt gct cag gcc tat gaa ctc gtc   96
Phe Gly Ile Lys Leu Asp Thr Tyr Phe Ala Gln Ala Tyr Glu Leu Val
            20                  25                  30

acc gga aag tcc atc gac tcc ttc gtc ttc cag gag ggc gtc acg cct  144
Thr Gly Lys Ser Ile Asp Ser Phe Val Phe Gln Glu Gly Val Thr Pro
        35                  40                  45

ctc tcg acc cag aga gag gtc gcc atg tgg act atc act tac ttc gtc  192
Leu Ser Thr Gln Arg Glu Val Ala Met Trp Thr Ile Thr Tyr Phe Val
    50                  55                  60

gtc atc ttt ggt ggt cgc cag atc atg aag agc cag gac gcc ttc aag  240
Val Ile Phe Gly Gly Arg Gln Ile Met Lys Ser Gln Asp Ala Phe Lys
65                  70                  75                  80

ctc aag ccc ctc ttc atc ctc cac aac ttc ctc ctg acg atc gcg tcc  288
Leu Lys Pro Leu Phe Ile Leu His Asn Phe Leu Leu Thr Ile Ala Ser
                85                  90                  95

gga tcg ctg ttg ctc ctg ttc atc gag aac ctg gtc ccc atc ctc gcc  336
Gly Ser Leu Leu Leu Leu Phe Ile Glu Asn Leu Val Pro Ile Leu Ala
            100                 105                 110

aga aac gga ctt ttc tac gcc atc tgc gac gac ggt gcc tgg acc cag  384
Arg Asn Gly Leu Phe Tyr Ala Ile Cys Asp Asp Gly Ala Trp Thr Gln
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       115                 120                 125

cgc ctc gag ctc ctc tac tac ctc aac tac ctg gtc aag tac tgg gag   432
Arg Leu Glu Leu Leu Tyr Tyr Leu Asn Tyr Leu Val Lys Tyr Trp Glu
    130                 135                 140

ttg gcc gac acc gtc ttt ttg gtc ctc aag aag aag cct ctt gag ttc   480
Leu Ala Asp Thr Val Phe Leu Val Leu Lys Lys Lys Pro Leu Glu Phe
145                 150                 155                 160

ctg cac tac ttc cac cac tcg atg acc atg gtt ctc tgc ttt gtc cag   528
Leu His Tyr Phe His His Ser Met Thr Met Val Leu Cys Phe Val Gln
                165                 170                 175

ctt gga gga tac act tca gtg tcc tgg gtc cct att acc ctc aac ttg   576
Leu Gly Gly Tyr Thr Ser Val Ser Trp Val Pro Ile Thr Leu Asn Leu
            180                 185                 190

act gtc cac gtc ttc atg tac tac tac tac atg cgc tcc gct gcc ggt   624
Thr Val His Val Phe Met Tyr Tyr Tyr Tyr Met Arg Ser Ala Ala Gly
        195                 200                 205

gtt cgc atc tgg tgg aag cag tac ttg acc act ctc cag atc gtc cag   672
Val Arg Ile Trp Trp Lys Gln Tyr Leu Thr Thr Leu Gln Ile Val Gln
    210                 215                 220

ttc gtt ctt gac ctc gga ttc atc tac ttc tgc gcc tac acc tac ttc   720
Phe Val Leu Asp Leu Gly Phe Ile Tyr Phe Cys Ala Tyr Thr Tyr Phe
225                 230                 235                 240

gcc ttc acc tac ttc ccc tgg gct ccc aac gtc ggc aag tgc gcc ggt   768
Ala Phe Thr Tyr Phe Pro Trp Ala Pro Asn Val Gly Lys Cys Ala Gly
                245                 250                 255

acc gag ggt gct gct ctc ttt ggc tgc gga ctc ctc tcc agc tat ctc   816
Thr Glu Gly Ala Ala Leu Phe Gly Cys Gly Leu Leu Ser Ser Tyr Leu
            260                 265                 270

ttg ctc ttt atc aac ttc tac cgc att acc tac aat gcc aag gcc aag   864
Leu Leu Phe Ile Asn Phe Tyr Arg Ile Thr Tyr Asn Ala Lys Ala Lys
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275 280 285

gca gcc aag gag cgt gga agc aac ttt acc ccc aag act gtc aag tcc 912
Ala Ala Lys Glu Arg Gly Ser Asn Phe Thr Pro Lys Thr Val Lys Ser
290 295 300

ggc gga tcg ccc aag aag ccc tcc aag agc aag cac atc taa 954
Gly Gly Ser Pro Lys Lys Pro Ser Lys Ser Lys His Ile
305 310 315

<210> 26
<211> 317
<212> PRT
<213> Mortierella alpina

<400> 26
Met Ala Ala Ala Ile Leu Asp Lys Val Asn Phe Gly Ile Asp Gln Pro
1 5 10 15

Phe Gly Ile Lys Leu Asp Thr Tyr Phe Ala Gln Ala Tyr Glu Leu Val
20 25 30

Thr Gly Lys Ser Ile Asp Ser Phe Val Phe Gln Glu Gly Val Thr Pro
35 40 45

Leu Ser Thr Gln Arg Glu Val Ala Met Trp Thr Ile Thr Tyr Phe Val
50 55 60

Val Ile Phe Gly Gly Arg Gln Ile Met Lys Ser Gln Asp Ala Phe Lys
65 70 75 80

Leu Lys Pro Leu Phe Ile Leu His Asn Phe Leu Leu Thr Ile Ala Ser
85 90 95

Gly Ser Leu Leu Leu Leu Phe Ile Glu Asn Leu Val Pro Ile Leu Ala
100 105 110

Arg Asn Gly Leu Phe Tyr Ala Ile Cys Asp Asp Gly Ala Trp Thr Gln
115 120 125

Arg Leu Glu Leu Leu Tyr Tyr Leu Asn Tyr Leu Val Lys Tyr Trp Glu
130 135 140

Leu Ala Asp Thr Val Phe Leu Val Leu Lys Lys Lys Pro Leu Glu Phe
145 150 155 160

Leu His Tyr Phe His His Ser Met Thr Met Val Leu Cys Phe Val Gln
165 170 175

Leu Gly Gly Tyr Thr Ser Val Ser Trp Val Pro Ile Thr Leu Asn Leu
180 185 190

Thr Val His Val Phe Met Tyr Tyr Tyr Tyr Met Arg Ser Ala Ala Gly
195 200 205

Val Arg Ile Trp Trp Lys Gln Tyr Leu Thr Thr Leu Gln Ile Val Gln
210 215 220

Phe Val Leu Asp Leu Gly Phe Ile Tyr Phe Cys Ala Tyr Thr Tyr Phe
225 230 235 240

Ala Phe Thr Tyr Phe Pro Trp Ala Pro Asn Val Gly Lys Cys Ala Gly
245 250 255

Thr Glu Gly Ala Ala Leu Phe Gly Cys Gly Leu Leu Ser Ser Tyr Leu
260 265 270

Leu Leu Phe Ile Asn Phe Tyr Arg Ile Thr Tyr Asn Ala Lys Ala Lys
275 280 285

Ala Ala Lys Glu Arg Gly Ser Asn Phe Thr Pro Lys Thr Val Lys Ser
290 295 300

Gly Gly Ser Pro Lys Lys Pro Ser Lys Ser Lys His Ile
305 310 315

<210> 27
<211> 1320

<212> DNA
<213> Thraustochytrium

<220>
<221> CDS
<222> (1)..(1320)
<223> Δ5-desaturase

<400> 27

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atg ggc aag ggc agc gag ggc cgc agc gcg gcg cgc gag atg acg gcc    48
Met Gly Lys Gly Ser Glu Gly Arg Ser Ala Ala Arg Glu Met Thr Ala
 1               5                  10                  15

gag gcg aac ggc gac aag cgg aaa acg att ctg atc gag ggc gtc ctg    96
Glu Ala Asn Gly Asp Lys Arg Lys Thr Ile Leu Ile Glu Gly Val Leu
            20                  25                  30

tac gac gcg acg aac ttt aag cac ccg ggc ggt tcg atc atc aac ttc   144
Tyr Asp Ala Thr Asn Phe Lys His Pro Gly Gly Ser Ile Ile Asn Phe
        35                  40                  45

ttg acc gag ggc gag gcc ggc gtg gac gcg acg cag gcg tac cgc gag   192
Leu Thr Glu Gly Glu Ala Gly Val Asp Ala Thr Gln Ala Tyr Arg Glu
    50                  55                  60

ttt cat cag cgg tcc ggc aag gcc gac aag tac ctc aag tcg ctg ccg   240
Phe His Gln Arg Ser Gly Lys Ala Asp Lys Tyr Leu Lys Ser Leu Pro
65                  70                  75                  80

aag ctg gat gcg tcc aag gtg gag tcg cgg ttc tcg gcc aaa gag cag   288
Lys Leu Asp Ala Ser Lys Val Glu Ser Arg Phe Ser Ala Lys Glu Gln
                85                  90                  95

gcg cgg cgc gac gcc atg acg cgc gac tac gcg gcc ttt cgc gag gag   336
Ala Arg Arg Asp Ala Met Thr Arg Asp Tyr Ala Ala Phe Arg Glu Glu
            100                 105                 110

ctc gtc gcc gag ggg tac ttt gac ccg tcg atc ccg cac atg att tac   384
Leu Val Ala Glu Gly Tyr Phe Asp Pro Ser Ile Pro His Met Ile Tyr
        115                 120                 125
```

```
cgc gtc gtg gag atc gtg gcg ctc ttc gcg ctc tcg ttc tgg ctc atg   432
Arg Val Val Glu Ile Val Ala Leu Phe Ala Leu Ser Phe Trp Leu Met
    130                 135                 140

tcc aag gcc tcg ccc acc tcg ctc gtg ctg ggc gtg gtg atg aac ggc   480
Ser Lys Ala Ser Pro Thr Ser Leu Val Leu Gly Val Val Met Asn Gly
145                 150                 155                 160

att gcg cag ggc cgc tgc ggc tgg gtc atg cac gag atg ggc cac ggg   528
Ile Ala Gln Gly Arg Cys Gly Trp Val Met His Glu Met Gly His Gly
                165                 170                 175

tcg ttc acg ggc gtc atc tgg ctc gac gac cgg atg tgc gag ttc ttc   576
Ser Phe Thr Gly Val Ile Trp Leu Asp Asp Arg Met Cys Glu Phe Phe
            180                 185                 190

tac ggc gtc ggc tgc ggc atg agc ggg cac tac tgg aag aac cag cac   624
Tyr Gly Val Gly Cys Gly Met Ser Gly His Tyr Trp Lys Asn Gln His
        195                 200                 205

agc aag cac cac gcc gcg ccc aac cgc ctc gag cac gat gtc gat ctc   672
Ser Lys His His Ala Ala Pro Asn Arg Leu Glu His Asp Val Asp Leu
    210                 215                 220

aac acg ctg ccc ctg gtc gcc ttt aac gag cgc gtc gtg cgc aag gtc   720
Asn Thr Leu Pro Leu Val Ala Phe Asn Glu Arg Val Val Arg Lys Val
225                 230                 235                 240

aag ccg gga tcg ctg ctg gcg ctc tgg ctg cgc gtg cag gcg tac ctc   768
Lys Pro Gly Ser Leu Leu Ala Leu Trp Leu Arg Val Gln Ala Tyr Leu
                245                 250                 255

ttt gcg ccc gtc tcg tgc ctg ctc atc ggc ctt ggc tgg acg ctc tac   816
Phe Ala Pro Val Ser Cys Leu Leu Ile Gly Leu Gly Trp Thr Leu Tyr
            260                 265                 270

ctg cac ccg cgc tac atg ctg cgc acc aag cgg cac atg gag ttc gtc   864
Leu His Pro Arg Tyr Met Leu Arg Thr Lys Arg His Met Glu Phe Val
        275                 280                 285
```

```
tgg atc ttc gcg cgc tac att ggc tgg ttc tcg ctc atg ggc gct ctc   912
Trp Ile Phe Ala Arg Tyr Ile Gly Trp Phe Ser Leu Met Gly Ala Leu
    290                 295                 300

ggc tac tcg ccg ggc acc tcg gtc ggg atg tac ctg tgc tcg ttc ggc   960
Gly Tyr Ser Pro Gly Thr Ser Val Gly Met Tyr Leu Cys Ser Phe Gly
305                 310                 315                 320

ctc ggc tgc att tac att ttc ctg cag ttc gcc gtc agc cac acg cac   1008
Leu Gly Cys Ile Tyr Ile Phe Leu Gln Phe Ala Val Ser His Thr His
                325                 330                 335

ctg ccg gtg acc aac ccg gag gac cag ctg cac tgg ctc gag tac gcg   1056
Leu Pro Val Thr Asn Pro Glu Asp Gln Leu His Trp Leu Glu Tyr Ala
            340                 345                 350

gcc gac cac acg gtg aac att agc acc aag tcc tgg ctc gtc acg tgg   1104
Ala Asp His Thr Val Asn Ile Ser Thr Lys Ser Trp Leu Val Thr Trp
        355                 360                 365

tgg atg tcg aac ctg aac ttt cag atc gag cac cac ctc ttc ccc acg   1152
Trp Met Ser Asn Leu Asn Phe Gln Ile Glu His His Leu Phe Pro Thr
    370                 375                 380

gcg ccg cag ttc cgc ttc aag gaa atc agt cct cgc gtc gag gcc ctc   1200
Ala Pro Gln Phe Arg Phe Lys Glu Ile Ser Pro Arg Val Glu Ala Leu
385                 390                 395                 400

ttc aag cgc cac aac ctc ccg tac tac gac ctg ccc tac acg agc gcg   1248
Phe Lys Arg His Asn Leu Pro Tyr Tyr Asp Leu Pro Tyr Thr Ser Ala
                405                 410                 415

gtc tcg acc acc ttt gcc aat ctt tat tcc gtc ggc cac tcg gtc ggc   1296
Val Ser Thr Thr Phe Ala Asn Leu Tyr Ser Val Gly His Ser Val Gly
            420                 425                 430

gcc gac acc aag aag cag gac tga                                   1320
Ala Asp Thr Lys Lys Gln Asp
        435                 440
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<210> 28
<211> 439
<212> PRT
<213> Thraustochytrium

<400> 28
Met Gly Lys Gly Ser Glu Gly Arg Ser Ala Ala Arg Glu Met Thr Ala
1 5 10 15

Glu Ala Asn Gly Asp Lys Arg Lys Thr Ile Leu Ile Glu Gly Val Leu
20 25 30

Tyr Asp Ala Thr Asn Phe Lys His Pro Gly Gly Ser Ile Ile Asn Phe
35 40 45

Leu Thr Glu Gly Glu Ala Gly Val Asp Ala Thr Gln Ala Tyr Arg Glu
50 55 60

Phe His Gln Arg Ser Gly Lys Ala Asp Lys Tyr Leu Lys Ser Leu Pro
65 70 75 80

Lys Leu Asp Ala Ser Lys Val Glu Ser Arg Phe Ser Ala Lys Glu Gln
85 90 95

Ala Arg Arg Asp Ala Met Thr Arg Asp Tyr Ala Ala Phe Arg Glu Glu
100 105 110

Leu Val Ala Glu Gly Tyr Phe Asp Pro Ser Ile Pro His Met Ile Tyr
115 120 125

Arg Val Val Glu Ile Val Ala Leu Phe Ala Leu Ser Phe Trp Leu Met
130 135 140

Ser Lys Ala Ser Pro Thr Ser Leu Val Leu Gly Val Val Met Asn Gly
145 150 155 160

Ile Ala Gln Gly Arg Cys Gly Trp Val Met His Glu Met Gly His Gly
165 170 175

Ser Phe Thr Gly Val Ile Trp Leu Asp Asp Arg Met Cys Glu Phe Phe
180 185 190

Tyr Gly Val Gly Cys Gly Met Ser Gly His Tyr Trp Lys Asn Gln His
195 200 205

Ser Lys His His Ala Ala Pro Asn Arg Leu Glu His Asp Val Asp Leu
210 215 220

Asn Thr Leu Pro Leu Val Ala Phe Asn Glu Arg Val Val Arg Lys Val
225 230 235 240

Lys Pro Gly Ser Leu Leu Ala Leu Trp Leu Arg Val Gln Ala Tyr Leu
245 250 255

Phe Ala Pro Val Ser Cys Leu Leu Ile Gly Leu Gly Trp Thr Leu Tyr
260 265 270

Leu His Pro Arg Tyr Met Leu Arg Thr Lys Arg His Met Glu Phe Val
275 280 285

Trp Ile Phe Ala Arg Tyr Ile Gly Trp Phe Ser Leu Met Gly Ala Leu
290 295 300

Gly Tyr Ser Pro Gly Thr Ser Val Gly Met Tyr Leu Cys Ser Phe Gly
305 310 315 320

Leu Gly Cys Ile Tyr Ile Phe Leu Gln Phe Ala Val Ser His Thr His
325 330 335

Leu Pro Val Thr Asn Pro Glu Asp Gln Leu His Trp Leu Glu Tyr Ala
340 345 350

Ala Asp His Thr Val Asn Ile Ser Thr Lys Ser Trp Leu Val Thr Trp
355 360 365

Trp Met Ser Asn Leu Asn Phe Gln Ile Glu His His Leu Phe Pro Thr
370 375 380

Ala Pro Gln Phe Arg Phe Lys Glu Ile Ser Pro Arg Val Glu Ala Leu
385 390 395 400

Phe Lys Arg His Asn Leu Pro Tyr Tyr Asp Leu Pro Tyr Thr Ser Ala
405 410 415

Val Ser Thr Thr Phe Ala Asn Leu Tyr Ser Val Gly His Ser Val Gly
420 425 430

Ala Asp Thr Lys Lys Gln Asp
435

<210> 29
<211> 957
<212> DNA
<213> Mortierella alpina

<220>
<221> CDS
<222> (1)..(957)
<223> Δ6-elongase

<400> 29
atg gag tcg att gcg cca ttc ctc cca tca aag atg ccg caa gat ctg 48
Met Glu Ser Ile Ala Pro Phe Leu Pro Ser Lys Met Pro Gln Asp Leu
1 5 10 15

ttt atg gac ctt gcc acc gct atc ggt gtc cgg gcc gcg ccc tat gtc 96
Phe Met Asp Leu Ala Thr Ala Ile Gly Val Arg Ala Ala Pro Tyr Val
20 25 30

gat cct ctc gag gcc gcg ctg gtg gcc cag gcc gag aag tac atc ccc 144
Asp Pro Leu Glu Ala Ala Leu Val Ala Gln Ala Glu Lys Tyr Ile Pro
35 40 45

acg att gtc cat cac acg cgt ggg ttc ctg gtc gcg gtg gag tcg cct 192
Thr Ile Val His His Thr Arg Gly Phe Leu Val Ala Val Glu Ser Pro
50 55 60

```
ttg gcc cgt gag ctg ccg ttg atg aac ccg ttc cac gtg ctg ttg atc   240
Leu Ala Arg Glu Leu Pro Leu Met Asn Pro Phe His Val Leu Leu Ile
 65                  70                  75                  80

gtg ctc gct tat ttg gtc acg gtc ttt gtg ggc atg cag atc atg aag   288
Val Leu Ala Tyr Leu Val Thr Val Phe Val Gly Met Gln Ile Met Lys
                85                  90                  95

aac ttt gag cgg ttc gag gtc aag acg ttt tcg ctc ctg cac aac ttt   336
Asn Phe Glu Arg Phe Glu Val Lys Thr Phe Ser Leu Leu His Asn Phe
            100                 105                 110

tgt ctg gtc tcg atc agc gcc tac atg tgc ggt ggg atc ctg tac gag   384
Cys Leu Val Ser Ile Ser Ala Tyr Met Cys Gly Gly Ile Leu Tyr Glu
        115                 120                 125

gct tat cag gcc aac tat gga ctg ttt gag aac gct gct gat cat acc   432
Ala Tyr Gln Ala Asn Tyr Gly Leu Phe Glu Asn Ala Ala Asp His Thr
    130                 135                 140

ttc aag ggt ctt cct atg gcc aag atg atc tgg ctc ttc tac ttc tcc   480
Phe Lys Gly Leu Pro Met Ala Lys Met Ile Trp Leu Phe Tyr Phe Ser
145                 150                 155                 160

aag atc atg gag ttt gtc gac acc atg atc atg gtc ctc aag aag aac   528
Lys Ile Met Glu Phe Val Asp Thr Met Ile Met Val Leu Lys Lys Asn
                165                 170                 175

aac cgc cag atc tcc ttc ttg cac gtt tac cac cac agc tcc atc ttc   576
Asn Arg Gln Ile Ser Phe Leu His Val Tyr His His Ser Ser Ile Phe
            180                 185                 190

acc atc tgg tgg ttg gtc acc ttt gtt gca ccc aac ggt gaa gcc tac   624
Thr Ile Trp Trp Leu Val Thr Phe Val Ala Pro Asn Gly Glu Ala Tyr
        195                 200                 205

ttc tct gct gcg ttg aac tcg ttc atc cat gtg atc atg tac ggc tac   672
Phe Ser Ala Ala Leu Asn Ser Phe Ile His Val Ile Met Tyr Gly Tyr
    210                 215                 220
```

```
tac ttc ttg tcg gcc ttg ggc ttc aag cag gtg tcg ttc atc aag ttc   720
Tyr Phe Leu Ser Ala Leu Gly Phe Lys Gln Val Ser Phe Ile Lys Phe
225                 230                 235                 240

tac atc acg cgc tcg cag atg aca cag ttc tgc atg atg tcg gtc cag   768
Tyr Ile Thr Arg Ser Gln Met Thr Gln Phe Cys Met Met Ser Val Gln
                245                 250                 255

tct tcc tgg gac atg tac gcc atg aag gtc ctt ggc cgc ccc gga tac   816
Ser Ser Trp Asp Met Tyr Ala Met Lys Val Leu Gly Arg Pro Gly Tyr
            260                 265                 270

ccc ttc ttc atc acg gct ctg ctt tgg ttc tac atg tgg acc atg ctc   864
Pro Phe Phe Ile Thr Ala Leu Leu Trp Phe Tyr Met Trp Thr Met Leu
        275                 280                 285

ggt ctc ttc tac aac ttt tac aga aag aac gcc aag ttg gcc aag cag   912
Gly Leu Phe Tyr Asn Phe Tyr Arg Lys Asn Ala Lys Leu Ala Lys Gln
    290                 295                 300

gcc aag gcc gac gct gcc aag gag aag gca agg aag ttg cag taa       957
Ala Lys Ala Asp Ala Ala Lys Glu Lys Ala Arg Lys Leu Gln
305                 310                 315
```

<210> 30
<211> 318
<212> PRT
<213> Mortierella alpina

<400> 30

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Met Glu Ser Ile Ala Pro Phe Leu Pro Ser Lys Met Pro Gln Asp Leu
  1               5                  10                  15

Phe Met Asp Leu Ala Thr Ala Ile Gly Val Arg Ala Ala Pro Tyr Val
             20                  25                  30

Asp Pro Leu Glu Ala Ala Leu Val Ala Gln Ala Glu Lys Tyr Ile Pro
         35                  40                  45
```

Thr Ile Val His His Thr Arg Gly Phe Leu Val Ala Val Glu Ser Pro
50 55 60

Leu Ala Arg Glu Leu Pro Leu Met Asn Pro Phe His Val Leu Leu Ile
65 70 75 80

Val Leu Ala Tyr Leu Val Thr Val Phe Val Gly Met Gln Ile Met Lys
85 90 95

Asn Phe Glu Arg Phe Glu Val Lys Thr Phe Ser Leu Leu His Asn Phe
100 105 110

Cys Leu Val Ser Ile Ser Ala Tyr Met Cys Gly Gly Ile Leu Tyr Glu
115 120 125

Ala Tyr Gln Ala Asn Tyr Gly Leu Phe Glu Asn Ala Ala Asp His Thr
130 135 140

Phe Lys Gly Leu Pro Met Ala Lys Met Ile Trp Leu Phe Tyr Phe Ser
145 150 155 160

Lys Ile Met Glu Phe Val Asp Thr Met Ile Met Val Leu Lys Lys Asn
165 170 175

Asn Arg Gln Ile Ser Phe Leu His Val Tyr His His Ser Ser Ile Phe
180 185 190

Thr Ile Trp Trp Leu Val Thr Phe Val Ala Pro Asn Gly Glu Ala Tyr
195 200 205

Phe Ser Ala Ala Leu Asn Ser Phe Ile His Val Ile Met Tyr Gly Tyr
210 215 220

Tyr Phe Leu Ser Ala Leu Gly Phe Lys Gln Val Ser Phe Ile Lys Phe
225 230 235 240

Tyr Ile Thr Arg Ser Gln Met Thr Gln Phe Cys Met Met Ser Val Gln
245 250 255

Ser Ser Trp Asp Met Tyr Ala Met Lys Val Leu Gly Arg Pro Gly Tyr

260 265 270

Pro Phe Phe Ile Thr Ala Leu Leu Trp Phe Tyr Met Trp Thr Met Leu
275 280 285

Gly Leu Phe Tyr Asn Phe Tyr Arg Lys Asn Ala Lys Leu Ala Lys Gln
290 295 300

Ala Lys Ala Asp Ala Ala Lys Glu Lys Ala Arg Lys Leu Gln
305 310 315

<210> 31
<211> 1374
<212> DNA
<213> Mortierella alpina

<220>
<221> CDS
<222> (1)..(1374)
<223> Δ6-desaturase

<400> 31
atg gct gct gct ccc agt gtg agg acg ttt act cgg gcc gag gtt ttg 48
Met Ala Ala Ala Pro Ser Val Arg Thr Phe Thr Arg Ala Glu Val Leu
1 5 10 15

aat gcc gag gct ctg aat gag ggc aag aag gat gcc gag gca ccc ttc 96
Asn Ala Glu Ala Leu Asn Glu Gly Lys Lys Asp Ala Glu Ala Pro Phe
20 25 30

ttg atg atc atc gac aac aag gtg tac gat gtt cgc gag ttc gtc cct 144
Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Glu Phe Val Pro
35 40 45

gat cat ccc ggt gga agt gtg att ctc acg cac gtt ggc aag gac ggc 192
Asp His Pro Gly Gly Ser Val Ile Leu Thr His Val Gly Lys Asp Gly
50 55 60

act gac gtc ttt gac act ttt cac ccc gag gct gct tgg gag act ctt 240

```
Thr Asp Val Phe Asp Thr Phe His Pro Glu Ala Ala Trp Glu Thr Leu
 65                  70                  75                  80

gcc aac ttt tac gtt ggt gat att gac gag agc gac cgc gat atc aag   288
Ala Asn Phe Tyr Val Gly Asp Ile Asp Glu Ser Asp Arg Asp Ile Lys
                85                  90                  95

aat gat gac ttt gcg gcc gag gtc cgc aag ctg cgt acc ttg ttc cag   336
Asn Asp Asp Phe Ala Ala Glu Val Arg Lys Leu Arg Thr Leu Phe Gln
            100                 105                 110

tct ctt ggt tac tac gat tct tcc aag gca tac tac gcc ttc aag gtc   384
Ser Leu Gly Tyr Tyr Asp Ser Ser Lys Ala Tyr Tyr Ala Phe Lys Val
        115                 120                 125

tcg ttc aac ctc tgc atc tgg ggt ttg tcg acg gtc att gtg gcc aag   432
Ser Phe Asn Leu Cys Ile Trp Gly Leu Ser Thr Val Ile Val Ala Lys
    130                 135                 140

tgg ggc cag acc tcg acc ctc gcc aac gtg ctc tcg gct gcg ctt ttg   480
Trp Gly Gln Thr Ser Thr Leu Ala Asn Val Leu Ser Ala Ala Leu Leu
145                 150                 155                 160

ggt ctg ttc tgg cag cag tgc gga tgg ttg gct cac gac ttt ttg cat   528
Gly Leu Phe Trp Gln Gln Cys Gly Trp Leu Ala His Asp Phe Leu His
                165                 170                 175

cac cag gtc ttc cag gac cgt ttc tgg ggt gat ctt ttc ggc gcc ttc   576
His Gln Val Phe Gln Asp Arg Phe Trp Gly Asp Leu Phe Gly Ala Phe
            180                 185                 190

ttg gga ggt gtc tgc cag ggc ttc tcg tcc tcg tgg tgg aag gac aag   624
Leu Gly Gly Val Cys Gln Gly Phe Ser Ser Ser Trp Trp Lys Asp Lys
        195                 200                 205

cac aac act cac cac gcc gcc ccc aac gtc cac ggc gag gat ccc gac   672
His Asn Thr His His Ala Ala Pro Asn Val His Gly Glu Asp Pro Asp
    210                 215                 220

att gac acc cac cct ctg ttg acc tgg agt gag cat gcg ttg gag atg   720
```

```
Ile Asp Thr His Pro Leu Leu Thr Trp Ser Glu His Ala Leu Glu Met
225                 230                 235                 240

ttc tcg gat gtc cca gat gag gag ctg acc cgc atg tgg tcg cgt ttc   768
Phe Ser Asp Val Pro Asp Glu Glu Leu Thr Arg Met Trp Ser Arg Phe
                245                 250                 255

atg gtc ctg aac cag acc tgg ttt tac ttc ccc att ctc tcg ttt gcc   816
Met Val Leu Asn Gln Thr Trp Phe Tyr Phe Pro Ile Leu Ser Phe Ala
            260                 265                 270

cgt ctc tcc tgg tgc ctc cag tcc att ctc ttt gtg ctg cct aac ggt   864
Arg Leu Ser Trp Cys Leu Gln Ser Ile Leu Phe Val Leu Pro Asn Gly
        275                 280                 285

cag gcc cac aag ccc tcg ggc gcg cgt gtg ccc atc tcg ttg gtc gag   912
Gln Ala His Lys Pro Ser Gly Ala Arg Val Pro Ile Ser Leu Val Glu
    290                 295                 300

cag ctg tcg ctt gcg atg cac tgg acc tgg tac ctc gcc acc atg ttc   960
Gln Leu Ser Leu Ala Met His Trp Thr Trp Tyr Leu Ala Thr Met Phe
305                 310                 315                 320

ctg ttc atc aag gat ccc gtc aac atg ctg gtg tac ttt ttg gtg tcg   1008
Leu Phe Ile Lys Asp Pro Val Asn Met Leu Val Tyr Phe Leu Val Ser
                325                 330                 335

cag gcg gtg tgc gga aac ttg ttg gcg atc gtg ttc tcg ctc aac cac   1056
Gln Ala Val Cys Gly Asn Leu Leu Ala Ile Val Phe Ser Leu Asn His
            340                 345                 350

aac ggt atg cct gtg atc tcg aag gag gag gcg gtc gat atg gat ttc   1104
Asn Gly Met Pro Val Ile Ser Lys Glu Glu Ala Val Asp Met Asp Phe
        355                 360                 365

ttc acg aag cag atc atc acg ggt cgt gat gtc cac ccg ggt cta ttt   1152
Phe Thr Lys Gln Ile Ile Thr Gly Arg Asp Val His Pro Gly Leu Phe
    370                 375                 380

gcc aac tgg ttc acg ggt gga ttg aac tat cag atc gag cac cac ttg   1200
```

```
Ala Asn Trp Phe Thr Gly Gly Leu Asn Tyr Gln Ile Glu His His Leu
385                 390                 395                 400

ttc cct tcg atg cct cgc cac aac ttt tca aag atc cag cct gct gtc   1248
Phe Pro Ser Met Pro Arg His Asn Phe Ser Lys Ile Gln Pro Ala Val
                405                 410                 415

gag acc ctg tgc aaa aag tac aat gtc cga tac cac acc acc ggt atg   1296
Glu Thr Leu Cys Lys Lys Tyr Asn Val Arg Tyr His Thr Thr Gly Met
            420                 425                 430

atc gag gga act gca gag gtc ttt agc cgt ctg aac gag gtc tcc aag   1344
Ile Glu Gly Thr Ala Glu Val Phe Ser Arg Leu Asn Glu Val Ser Lys
        435                 440                 445

gct gcc tcc aag atg ggt aag gcg cag taa                          1374
Ala Ala Ser Lys Met Gly Lys Ala Gln
    450                 455
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<210> 32
<211> 457
<212> PRT
<213> Mortierella alpina

<400> 32

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Met Ala Ala Ala Pro Ser Val Arg Thr Phe Thr Arg Ala Glu Val Leu
  1               5                  10                  15

Asn Ala Glu Ala Leu Asn Glu Gly Lys Lys Asp Ala Glu Ala Pro Phe
            20                  25                  30

Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Glu Phe Val Pro
        35                  40                  45

Asp His Pro Gly Gly Ser Val Ile Leu Thr His Val Gly Lys Asp Gly
    50                  55                  60

Thr Asp Val Phe Asp Thr Phe His Pro Glu Ala Ala Trp Glu Thr Leu
65                  70                  75                  80
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Ala Asn Phe Tyr Val Gly Asp Ile Asp Glu Ser Asp Arg Asp Ile Lys
85 90 95

Asn Asp Asp Phe Ala Ala Glu Val Arg Lys Leu Arg Thr Leu Phe Gln
100 105 110

Ser Leu Gly Tyr Tyr Asp Ser Ser Lys Ala Tyr Tyr Ala Phe Lys Val
115 120 125

Ser Phe Asn Leu Cys Ile Trp Gly Leu Ser Thr Val Ile Val Ala Lys
130 135 140

Trp Gly Gln Thr Ser Thr Leu Ala Asn Val Leu Ser Ala Ala Leu Leu
145 150 155 160

Gly Leu Phe Trp Gln Gln Cys Gly Trp Leu Ala His Asp Phe Leu His
165 170 175

His Gln Val Phe Gln Asp Arg Phe Trp Gly Asp Leu Phe Gly Ala Phe
180 185 190

Leu Gly Gly Val Cys Gln Gly Phe Ser Ser Ser Trp Trp Lys Asp Lys
195 200 205

His Asn Thr His His Ala Ala Pro Asn Val His Gly Glu Asp Pro Asp
210 215 220

Ile Asp Thr His Pro Leu Leu Thr Trp Ser Glu His Ala Leu Glu Met
225 230 235 240

Phe Ser Asp Val Pro Asp Glu Glu Leu Thr Arg Met Trp Ser Arg Phe
245 250 255

Met Val Leu Asn Gln Thr Trp Phe Tyr Phe Pro Ile Leu Ser Phe Ala
260 265 270

Arg Leu Ser Trp Cys Leu Gln Ser Ile Leu Phe Val Leu Pro Asn Gly
275 280 285

Gln Ala His Lys Pro Ser Gly Ala Arg Val Pro Ile Ser Leu Val Glu
290 295 300

Gln Leu Ser Leu Ala Met His Trp Thr Trp Tyr Leu Ala Thr Met Phe
305 310 315 320

Leu Phe Ile Lys Asp Pro Val Asn Met Leu Val Tyr Phe Leu Val Ser
325 330 335

Gln Ala Val Cys Gly Asn Leu Leu Ala Ile Val Phe Ser Leu Asn His
340 345 350

Asn Gly Met Pro Val Ile Ser Lys Glu Glu Ala Val Asp Met Asp Phe
355 360 365

Phe Thr Lys Gln Ile Ile Thr Gly Arg Asp Val His Pro Gly Leu Phe
370 375 380

Ala Asn Trp Phe Thr Gly Gly Leu Asn Tyr Gln Ile Glu His His Leu
385 390 395 400

Phe Pro Ser Met Pro Arg His Asn Phe Ser Lys Ile Gln Pro Ala Val
405 410 415

Glu Thr Leu Cys Lys Lys Tyr Asn Val Arg Tyr His Thr Thr Gly Met
420 425 430

Ile Glu Gly Thr Ala Glu Val Phe Ser Arg Leu Asn Glu Val Ser Lys
435 440 445

Ala Ala Ser Lys Met Gly Lys Ala Gln
450 455

<210> 33
<211> 3598
<212> DNA
<213> Unknown

<220>

<223> Sequence constitutes a plant
promoter-terminator expression cassette in vector
pUC19

<400> 33
tcgcgcgttt cggtgatgac ggtgaaaacc tctgacacat gcagctcccg gagacggtca 60

cagcttgtct gtaagcggat gccgggagca gacaagcccg tcagggcgcg tcagcgggtg 120

ttggcgggtg tcggggctgg cttaactatg cggcatcaga gcagattgta ctgagagtgc 180

accatatgcg gtgtgaaata ccgcacagat gcgtaaggag aaaataccgc atcaggcgcc 240

attcgccatt caggctgcgc aactgttggg aagggcgatc ggtgcgggcc tcttcgctat 300

tacgccagct ggcgaaaggg ggatgtgctg caaggcgatt aagttgggta acgccagggt 360

tttcccagtc acgacgttgt aaaacgacgg ccagtgaatt cggcgcgccg agctcctcga 420

gcaaatttac acattgccac taaacgtcta aacccttgta atttgttttt gttttactat 480

gtgtgttatg tatttgattt gcgataaatt tttatatttg gtactaaatt tataacacct 540

tttatgctaa cgtttgccaa cacttagcaa tttgcaagtt gattaattga ttctaaatta 600

tttttgtctt ctaaatacat atactaatca actggaaatg taaatatttg ctaatatttc 660

tactatagga gaattaaagt gagtgaatat ggtaccacaa ggtttggaga tttaattgtt 720

gcaatgctgc atggatggca tatacaccaa acattcaata attcttgagg ataataatgg 780

taccacacaa gatttgaggt gcatgaacgt cacgtggaca aaaggtttag taatttttca 840

agacaacaat gttaccacac acaagttttg aggtgcatgc atggatgccc tgtggaaagt 900

ttaaaaatat tttggaaatg atttgcatgg aagccatgtg taaaaccatg acatccactt 960

ggaggatgca ataatgaaga aaactacaaa tttacatgca actagttatg catgtagtct 1020

atataatgag gattttgcaa tactttcatt catacacact cactaagttt tacacgatta 1080

taatttcttc atagccagcc caccgcggtg ggcggccgcc tgcagtctag aaggcctcct 1140

gctttaatga gatatgcgag acgcctatga tcgcatgata tttgctttca attctgttgt 1200

gcacgttgta aaaaacctga gcatgtgtag ctcagatcct taccgccggt ttcggttcat 1260

tctaatgaat atatcacccg ttactatcgt atttttatga ataatattct ccgttcaatt 1320

tactgattgt ccgtcgacga attcgagctc ggcgcgccaa gcttggcgta atcatggtca 1380

tagctgtttc ctgtgtgaaa ttgttatccg ctcacaattc cacacaacat acgagccgga 1440

agcataaagt gtaaagcctg gggtgcctaa tgagtgagct aactcacatt aattgcgttg 1500

cgctcactgc ccgctttcca gtcgggaaac ctgtcgtgcc agctgcatta atgaatcggc 1560

caacgcgcgg ggagaggcgg tttgcgtatt gggcgctctt ccgcttcctc gctcactgac 1620

tcgctgcgct cggtcgttcg gctgcggcga gcggtatcag ctcactcaaa ggcggtaata 1680

cggttatcca cagaatcagg ggataacgca ggaaagaaca tgtgagcaaa aggccagcaa 1740

aaggccagga accgtaaaaa ggccgcgttg ctggcgtttt tccataggct ccgccccct 1800

gacgagcatc acaaaaatcg acgctcaagt cagaggtggc gaaacccgac aggactataa 1860

agataccagg cgtttccccc tggaagctcc ctcgtgcgct ctcctgttcc gaccctgccg 1920

cttaccggat acctgtccgc ctttctccct tcgggaagcg tggcgctttc tcatagctca 1980

cgctgtaggt atctcagttc ggtgtaggtc gttcgctcca agctgggctg tgtgcacgaa 2040

ccccccgttc agcccgaccg ctgcgcctta tccggtaact atcgtcttga gtccaacccg 2100

gtaagacacg acttatcgcc actggcagca gccactggta acaggattag cagagcgagg 2160

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tgaagcattt atcagggtta ttgtctcatg agcggataca tatttgaatg tatttagaaa 3480

aataaacaaa taggggttcc gcgcacattt ccccgaaaag tgccacctga cgtctaagaa 3540

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<210> 34
<211> 3590
<212> DNA
<213> Unknown

<220>
<223> Sequence constitutes a plant
promoter-terminator expression cassette in vector
pUC19

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ttggcgggtg tcggggctgg cttaactatg cggcatcaga gcagattgta ctgagagtgc 180

accatatgcg gtgtgaaata ccgcacagat gcgtaaggag aaaataccgc atcaggcgcc 240

attcgccatt caggctgcgc aactgttggg aagggcgatc ggtgcgggcc tcttcgctat 300

tacgccagct ggcgaaaggg ggatgtgctg caaggcgatt aagttgggta acgccagggt 360

tttcccagtc acgacgttgt aaaacgacgg ccagtgaatt cggcgcgccg agctcctcga 420

gcaaatttac acattgccac taaacgtcta aacccttgta atttgttttt gttttactat 480

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tactatagga gaattaaagt gagtgaatat ggtaccacaa ggtttggaga tttaattgtt 720

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atataatgag gattttgcaa tactttcatt catacacact cactaagttt tacacgatta 1080

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aagggcgaca cggaaatgtt gaatactcat actcttcctt tttcaatatt attgaagcat 3420

ttatcagggt tattgtctca tgagcggata catatttgaa tgtatttaga aaaataaaca 3480

aataggggtt ccgcgcacat ttccccgaaa agtgccacct gacgtctaag aaaccattat 3540

tatcatgaca ttaacctata aaaataggcg tatcacgagg ccctttcgtc 3590

<210> 35
<211> 3584
<212> DNA
<213> Unknown

<220>
<223> Sequence constitutes a plant
promoter-terminator expression cassette in vector
pUC19

<400> 35
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ttggcgggtg tcggggctgg cttaactatg cggcatcaga gcagattgta ctgagagtgc 180

accatatgcg gtgtgaaata ccgcacagat gcgtaaggag aaaataccgc atcaggcgcc 240

attcgccatt caggctgcgc aactgttggg aagggcgatc ggtgcgggcc tcttcgctat 300

tacgccagct ggcgaaaggg ggatgtgctg caaggcgatt aagttgggta acgccagggt 360

tttcccagtc acgacgttgt aaaacgacgg ccagtgaatt cggcgcgccg agctcctcga 420

gcaaatttac acattgccac taaacgtcta aacccttgta atttgttttt gttttactat 480

gtgtgtttat g tatttgattt gcgataaatt tttatatttg gtactaaatt tataacacct 540

tttatgctaa cgtttgccaa cacttagcaa tttgcaagtt gattaattga ttctaaatta 600

tttttgtctt ctaaatacat atactaatca actggaaatg taaatatttg ctaatatttc 660

tactatagga gaattaaagt gagtgaatat ggtaccacaa ggtttggaga tttaattgtt 720

gcaatgctgc atggatggca tatacaccaa acattcaata attcttgagg ataataatgg 780

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ttaaaaatat tttggaaatg atttgcatgg aagccatgtg taaaaccatg acatccactt 960

ggaggatgca ataatgaaga aaactacaaa tttacatgca actagttatg catgtagtct 1020

atataatgag gattttgcaa tactttcatt catacacact cactaagttt tacacgatta 1080

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gagatatgcg agacgcctat gatcgcatga tatttgcttt caattctgtt gtgcacgttg 1200

taaaaaacct gagcatgtgt agctcagatc cttaccgccg gtttcggttc attctaatga 1260

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cagcgtttct gggtgagcaa aaacaggaag gcaaaatgcc gcaaaaaagg gaataagggc 3360

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gggttattgt ctcatgagcg gatacatatt tgaatgtatt tagaaaaata aacaaatagg 3480

ggttccgcgc acatttcccc gaaaagtgcc acctgacgtc taagaaacca ttattatcat 3540

gacattaacc tataaaaata ggcgtatcac gaggcccttt cgtc 3584

<210> 36
<211> 4507
<212> DNA
<213> Unknown

<220>
<223> Sequence constitutes a plant
promoter-terminator expression cassette in vector
pUC19

<400> 36

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ttggcgggtg tcggggctgg cttaactatg cggcatcaga gcagattgta ctgagagtgc 180

accatatgcg gtgtgaaata ccgcacagat gcgtaaggag aaaataccgc atcaggcgcc 240

attcgccatt caggctgcgc aactgttggg aagggcgatc ggtgcgggcc tcttcgctat 300

tacgccagct ggcgaaaggg ggatgtgctg caaggcgatt aagttgggta acgccagggt 360

tttcccagtc acgacgttgt aaaacgacgg ccagtgaatt cggcgcgccg agctcctcga 420

gcaaatttac acattgccac taaacgtcta aacccttgta atttgttttt gttttaatat 480

gtgtgttatg tatttgattt gcgataaatt tttatatttg gtactaaatt tataacacct 540

tttatgctaa cgtttgccaa cacttagcaa tttgcaagtt gattaattga ttctaaatta 600

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gcacgttgta aaaaacctga gcatgtgtag ctcagatcct taccgccggt ttcggttcat 1260

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tttcgtc 4507

<210> 37
<211> 5410
<212> DNA
<213> Unknown

<220>

<223> Sequence constitutes a plant promoter-terminator expression cassette in vector pUC19

<400> 37

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Gly Val Glu Leu Thr Asp Thr Pro Thr Thr Lys Gly Leu Pro Leu Val
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Asp Ser Pro Thr Pro Ile Val Leu Gly Val Ser Val Tyr Leu Thr Ile
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gtc att gga ggg ctt ttg tgg ata aag gcc agg gat ctg aaa ccg cgc   11764
Val Ile Gly Gly Leu Leu Trp Ile Lys Ala Arg Asp Leu Lys Pro Arg
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Ala Ser Glu Pro Phe Leu Leu Gln Ala Leu Val Leu Val His Asn Leu
75                  80                  85                  90

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Phe Cys Phe Ala Leu Ser Leu Tyr Met Cys Val Gly Ile Ala Tyr Gln
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Ala Ile Thr Trp Arg Tyr Ser Leu Trp Gly Asn Ala Tyr Asn Pro Lys
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cat aaa gag atg gcg att ctg gta tac ttg ttc tac atg tct aag tac   11956
His Lys Glu Met Ala Ile Leu Val Tyr Leu Phe Tyr Met Ser Lys Tyr
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gtg gaa ttc atg gat acc gtt atc atg ata ctg aag cgc agc acc agg   12004
Val Glu Phe Met Asp Thr Val Ile Met Ile Leu Lys Arg Ser Thr Arg
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caa ata agc ttc ctc cac gtt tat cat cat tct tca att tcc ctc att   12052
Gln Ile Ser Phe Leu His Val Tyr His His Ser Ser Ile Ser Leu Ile
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Ala Ser Met Ser Leu Phe Ser Asp Phe Phe Ser Tyr Val Ser Ser Thr
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Val Gly Ser Trp Ser Val His Ser Ile Gln Pro Leu Lys Arg Leu Thr
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Ser Lys Lys Arg Val Ser Glu Ser Ala Ala Val Gln Cys Ile Ser Ala
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Glu Val Gln Arg Asn Ser Ser Thr Gln Gly Thr Ala Glu Ala Leu Ala
            365                 370                 375

gaa tca gtc gtg aag ccc acg aga cga agg tca tct cag tgg aaq aag   13618
Glu Ser Val Val Lys Pro Thr Arg Arg Arg Ser Ser Gln Trp Lys Lys
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tcg aca cac ccc cta tca gaa gta gca gta cac aac aag cca agc gat   13666
Ser Thr His Pro Leu Ser Glu Val Ala Val His Asn Lys Pro Ser Asp
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tgc tgg att gtt gta aaa aac aag gtg tat gat gtt tcc aat ttt gcg   13714
Cys Trp Ile Val Val Lys Asn Lys Val Tyr Asp Val Ser Asn Phe Ala
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Asp Glu His Pro Gly Gly Ser Val Ile Ser Thr Tyr Phe Gly Arg Asp
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ggc aca gat gtt ttc tct agt ttt cat gca gct tct aca tgg aaa att   13810
Gly Thr Asp Val Phe Ser Ser Phe His Ala Ala Ser Thr Trp Lys Ile
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Leu Gln Asp Phe Tyr Ile Gly Asp Val Glu Arg Val Glu Pro Thr Pro
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Glu Leu Leu Lys Asp Phe Arg Glu Met Arg Ala Leu Phe Leu Arg Glu
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Gln Gln Cys Gly Trp Leu Ser His Asp Phe Leu His Asn Gln Val Phe
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Glu Thr Arg Trp Leu Asn Glu Val Val Gly Tyr Val Ile Gly Asn Ala
555 560 565

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Ser Trp Arg Tyr Thr Ser Thr Ala Val Leu Ser Pro Val Asp Arg Leu
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Thr Glu Leu Met Ser Gly Met Leu Leu Gly Phe Val Phe Val Leu Ser
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cac aat ggg atg gag gtt tat aat tcg tct aaa gaa ttc gtg agt gca   14626
His Asn Gly Met Glu Val Tyr Asn Ser Ser Lys Glu Phe Val Ser Ala
    715                 720                 725

cag atc gta tcc aca cgg gat atc aaa gga aac ata ttc aac gac tgg   14674
Gln Ile Val Ser Thr Arg Asp Ile Lys Gly Asn Ile Phe Asn Asp Trp
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Phe Thr Gly Gly Leu Asn Arg Gln Ile Glu His His Leu Phe Pro Thr
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Met Pro Arg His Asn Leu Asn Lys Ile Ala Pro Arg Val Glu Val Phe
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Cys Lys Lys His Gly Leu Val Tyr Glu Asp Val Ser Ile Ala Thr Gly
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Thr Cys Lys Val Leu Lys Ala Leu Lys Glu Val Ala Glu Ala Ala Ala
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Trp Ile Lys Ala Arg Asp Leu Lys Pro Arg Ala Ser Glu Pro Phe Leu
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Leu Gln Ala Leu Val Leu Val His Asn Leu Phe Cys Phe Ala Leu Ser
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Leu Tyr Met Cys Val Gly Ile Ala Tyr Gln Ala Ile Thr Trp Arg Tyr
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Ser Leu Trp Gly Asn Ala Tyr Asn Pro Lys His Lys Glu Met Ala Ile
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Leu Val Tyr Leu Phe Tyr Met Ser Lys Tyr Val Glu Phe Met Asp Thr
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Val Ile Met Ile Leu Lys Arg Ser Thr Arg Gln Ile Ser Phe Leu His
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Val Tyr His His Ser Ser Ile Ser Leu Ile Trp Trp Ala Ile Ala His
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His Ala Pro Gly Gly Glu Ala Tyr Trp Ser Ala Ala Leu Asn Ser Gly
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Val His Val Leu Met Tyr Ala Tyr Tyr Phe Leu Ala Ala Cys Leu Arg
195 200 205

Ser Ser Pro Lys Leu Lys Asn Lys Tyr Leu Phe Trp Gly Arg Tyr Leu
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Thr Gln Phe Gln Met Phe Gln Phe Met Leu Asn Leu Val Gln Ala Tyr
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Tyr Asp Met Lys Thr Asn Ala Pro Tyr Pro Gln Trp Leu Ile Lys Ile
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His Asn Lys Pro Ser Asp Cys Trp Ile Val Val Lys Asn Lys Val Tyr
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Ala Leu Phe Leu Arg Glu Gln Leu Phe Lys Ser Ser Lys Leu Tyr Tyr
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Ser Lys Asp Ile Leu Ala Thr Val Glu Asn Lys Thr Phe Leu Arg Ile
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Lys Glu Phe Val Ser Ala Gln Ile Val Ser Thr Arg Asp Ile Lys Gly
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ttg gag aag gga act gtt ctg ttt cac tac ttt tgg ttc gtc ggg aca 14482
Leu Glu Lys Gly Thr Val Leu Phe His Tyr Phe Trp Phe Val Gly Thr
670 675 680

gcg tgc tat ctt ctc cct ggt tgg aag cca tta gta tgg atg gcg gtg 14530
Ala Cys Tyr Leu Leu Pro Gly Trp Lys Pro Leu Val Trp Met Ala Val

685 690 695

act gag ctc atg tcc ggc atg ctg ctg ggc ttt gta ttt gta ctt agc 14578
Thr Glu Leu Met Ser Gly Met Leu Leu Gly Phe Val Phe Val Leu Ser
700 705 710

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His Asn Gly Met Glu Val Tyr Asn Ser Ser Lys Glu Phe Val Ser Ala
715 720 725

cag atc gta tcc aca cgg gat atc aaa gga aac ata ttc aac gac tgg 14674
Gln Ile Val Ser Thr Arg Asp Ile Lys Gly Asn Ile Phe Asn Asp Trp
730 735 740 745

ttc act ggt ggc ctt aac agg caa ata gag cat cat ctt ttc cca aca 14722
Phe Thr Gly Gly Leu Asn Arg Gln Ile Glu His His Leu Phe Pro Thr
750 755 760

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Met Pro Arg His Asn Leu Asn Lys Ile Ala Pro Arg Val Glu Val Phe
765 770 775

tgt aag aaa cac ggt ctg gtg tac gaa gac gta tct att gct acc ggc 14818
Cys Lys Lys His Gly Leu Val Tyr Glu Asp Val Ser Ile Ala Thr Gly
780 785 790

act tgc aag gtt ttg aaa gca ttg aag gaa gtc gcg gag gct gcg gca 14866
Thr Cys Lys Val Leu Lys Ala Leu Lys Glu Val Ala Glu Ala Ala Ala
795 800 805

gag cag cat gct acc acc agt taa gctagcgtta accctgcttt aatgagatat 14920
Glu Gln His Ala Thr Thr Ser
810 815

gcgagacgcc tatgatcgca tgatatttgc tttcaattct gttgtgcacg ttgtaaaaaa 14980

cctgagcatg tgtagctcag atccttaccg ccggtttcgg ttcattctaa tgaatatatc 15040

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gtttaaaaat attttggaaa tgatttgcat ggaagccatg tgtaaaacca tgacatccac 15640

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tataatttct tcatagccag cagatctaaa atg gct ccg gat gcg gat aag ctt 15814
Met Ala Pro Asp Ala Asp Lys Leu
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cga caa cgc cag acg act gcg gta gcg aag cac aat gct gct acc ata 15862
Arg Gln Arg Gln Thr Thr Ala Val Ala Lys His Asn Ala Ala Thr Ile
830 835 840

tcg acg cag gaa cgc ctt tgc agt ctg tct tcg ctc aaa ggc gaa gaa 15910
Ser Thr Gln Glu Arg Leu Cys Ser Leu Ser Ser Leu Lys Gly Glu Glu
845 850 855

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Val Cys Ile Asp Gly Ile Ile Tyr Asp Leu Gln Ser Phe Asp His Pro
860 865 870

ggg ggt gaa acg atc aaa atg ttt ggt ggc aac gat gtc act gta cag 16006
Gly Gly Glu Thr Ile Lys Met Phe Gly Gly Asn Asp Val Thr Val Gln

875 880 885

tac aag atg att cac ccg tac cat acc gag aag cat ttg gaa aag atg 16054
Tyr Lys Met Ile His Pro Tyr His Thr Glu Lys His Leu Glu Lys Met
890 895 900 905

aag cgt gtc ggc aag gtg acg gat ttc gtc tgc gag tac aag ttc gat 16102
Lys Arg Val Gly Lys Val Thr Asp Phe Val Cys Glu Tyr Lys Phe Asp
910 915 920

acc gaa ttt gaa cgc gaa atc aaa cga gaa gtc ttc aag att gtg cga 16150
Thr Glu Phe Glu Arg Glu Ile Lys Arg Glu Val Phe Lys Ile Val Arg
925 930 935

cga ggc aag gat ttc ggt act ttg gga tgg ttc ttc cgt gcg ttt tgc 16198
Arg Gly Lys Asp Phe Gly Thr Leu Gly Trp Phe Phe Arg Ala Phe Cys
940 945 950

tac att gcc att ttc ttc tac ctg cag tac cat tgg gtc acc acg gga 16246
Tyr Ile Ala Ile Phe Phe Tyr Leu Gln Tyr His Trp Val Thr Thr Gly
955 960 965

acc tct tgg ctg ctg gcc gtg gcc tac gga atc tcc caa gcg atg att 16294
Thr Ser Trp Leu Leu Ala Val Ala Tyr Gly Ile Ser Gln Ala Met Ile
970 975 980 985

ggc atg aat gtc cag cac gat gcc aac cac ggg gcc acc tcc aag cgt 16342
Gly Met Asn Val Gln His Asp Ala Asn His Gly Ala Thr Ser Lys Arg
990 995 1000

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Pro Trp Val Asn Asp Met Leu Gly Leu Gly Ala Asp Phe Ile Gly Gly
1005 1010 1015

tcc aag tgg ctc tgg cag gaa caa cac tgg acc cac cac gct tac acc 16438
Ser Lys Trp Leu Trp Gln Glu Gln His Trp Thr His His Ala Tyr Thr
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aat cac gcc gag atg gat ccc gat agc ttt ggt gcc gaa cca atg ctc 16486
Asn His Ala Glu Met Asp Pro Asp Ser Phe Gly Ala Glu Pro Met Leu

1035 1040 1045

cta ttc aac gac tat ccc ttg gat cat ccc gct cgt acc tgg cta cat 16534
Leu Phe Asn Asp Tyr Pro Leu Asp His Pro Ala Arg Thr Trp Leu His
1050 1055 1060 1065

cgc ttt caa gca ttc ttt tac atg ccc gtc ttg gct gga tac tgg ttg 16582
Arg Phe Gln Ala Phe Phe Tyr Met Pro Val Leu Ala Gly Tyr Trp Leu
1070 1075 1080

tcc gct gtc ttc aat cca caa att ctt gac ctc cag caa cgc ggc gca 16630
Ser Ala Val Phe Asn Pro Gln Ile Leu Asp Leu Gln Gln Arg Gly Ala
1085 1090 1095

ctt tcc gtc ggt atc cgt ctc gac aac gct ttc att cac tcg cga cgc 16678
Leu Ser Val Gly Ile Arg Leu Asp Asn Ala Phe Ile His Ser Arg Arg
1100 1105 1110

aag tat gcg gtt ttc tgg cgg gct gtg tac att gcg gtg aac gtg att 16726
Lys Tyr Ala Val Phe Trp Arg Ala Val Tyr Ile Ala Val Asn Val Ile
1115 1120 1125

gct ccg ttt tac aca aac tcc ggc ctc gaa tgg tcc tgg cgt gtc ttt 16774
Ala Pro Phe Tyr Thr Asn Ser Gly Leu Glu Trp Ser Trp Arg Val Phe
1130 1135 1140 1145

gga aac atc atg ctc atg ggt gtg gcg gaa tcg ctc gcg ctg gcg gtc 16822
Gly Asn Ile Met Leu Met Gly Val Ala Glu Ser Leu Ala Leu Ala Val
1150 1155 1160

ctg ttt tcg ttg tcg cac aat ttc gaa tcc gcg gat cgc gat ccg acc 16870
Leu Phe Ser Leu Ser His Asn Phe Glu Ser Ala Asp Arg Asp Pro Thr
1165 1170 1175

gcc cca ctg aaa aag acg gga gaa cca gtc gac tgg ttc aag aca cag 16918
Ala Pro Leu Lys Lys Thr Gly Glu Pro Val Asp Trp Phe Lys Thr Gln
1180 1185 1190

gtc gaa act tcc tgc act tac ggt gga ttc ctt tcc ggt tgc ttc acg 16966
Val Glu Thr Ser Cys Thr Tyr Gly Gly Phe Leu Ser Gly Cys Phe Thr

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gga ggt ctc aac ttt cag gtt gaa cac cac ttg ttc cca cgc atg agc   17014
Gly Gly Leu Asn Phe Gln Val Glu His His Leu Phe Pro Arg Met Ser
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agc gct tgg tat ccc tac att gcc ccc aag gtc cgc gaa att tgc gcc   17062
Ser Ala Trp Tyr Pro Tyr Ile Ala Pro Lys Val Arg Glu Ile Cys Ala
                1230                1235                1240

aaa cac ggc gtc cac tac gcc tac tac ccg tgg atc cac caa aac ttt   17110
Lys His Gly Val His Tyr Ala Tyr Tyr Pro Trp Ile His Gln Asn Phe
        1245                1250                1255

ctc tcc acc gtc cgc tac atg cac gcg gcc ggg acc ggt gcc aac tgg   17158
Leu Ser Thr Val Arg Tyr Met His Ala Ala Gly Thr Gly Ala Asn Trp
    1260                1265                1270

cgc cag atg gcc aga gaa aat ccc ttg acc gga cgg gcg taa           17200
Arg Gln Met Ala Arg Glu Asn Pro Leu Thr Gly Arg Ala
1275                1280                1285

agatctgccg gcatcgatcc cgggccatgg cctgctttaa tgagatatgc gagacgccta 17260

tgatcgcatg atatttgctt tcaattctgt tgtgcacgtt gtaaaaaacc tgagcatgtg 17320

tagctcagat ccttaccgcc ggtttcggtt cattctaatg aatatatcac ccgttactat 17380

cgtatttta tgaataatat tctccgttca atttactgat tgtccgtcga cgagctcggc 17440

gcgcctctag aggatcgatg aattcagatc ggctgagtgg ctccttcaac gttgcggttc 17500

tgtcagttcc aaacgtaaaa cggcttgtcc cgcgtcatcg gcgggggtca taacgtgact 17560

cccttaattc tccgctcatg atcagattgt cgtttcccgc cttcagttta aactatcagt 17620

gtttgacagg atatattggc gggtaaacct aagagaaaag agcgtttatt agaataatcg 17680

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Gln Gly Val Asn Ala Leu Leu Gly Ser Phe Gly Val Glu Leu Thr Asp
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Thr Pro Thr Thr Lys Gly Leu Pro Leu Val Asp Ser Pro Thr Pro Ile
35 40 45

Val Leu Gly Val Ser Val Tyr Leu Thr Ile Val Ile Gly Gly Leu Leu
50 55 60

Trp Ile Lys Ala Arg Asp Leu Lys Pro Arg Ala Ser Glu Pro Phe Leu
65 70 75 80

Leu Gln Ala Leu Val Leu Val His Asn Leu Phe Cys Phe Ala Leu Ser
85 90 95

Leu Tyr Met Cys Val Gly Ile Ala Tyr Gln Ala Ile Thr Trp Arg Tyr
100 105 110

Ser Leu Trp Gly Asn Ala Tyr Asn Pro Lys His Lys Glu Met Ala Ile
115 120 125

Leu Val Tyr Leu Phe Tyr Met Ser Lys Tyr Val Glu Phe Met Asp Thr
130 135 140

Val Ile Met Ile Leu Lys Arg Ser Thr Arg Gln Ile Ser Phe Leu His
145 150 155 160

Val Tyr His His Ser Ser Ile Ser Leu Ile Trp Trp Ala Ile Ala His

165 170 175

His Ala Pro Gly Gly Glu Ala Tyr Trp Ser Ala Ala Leu Asn Ser Gly
180 185 190

Val His Val Leu Met Tyr Ala Tyr Tyr Phe Leu Ala Ala Cys Leu Arg
195 200 205

Ser Ser Pro Lys Leu Lys Asn Lys Tyr Leu Phe Trp Gly Arg Tyr Leu
210 215 220

Thr Gln Phe Gln Met Phe Gln Phe Met Leu Asn Leu Val Gln Ala Tyr
225 230 235 240

Tyr Asp Met Lys Thr Asn Ala Pro Tyr Pro Gln Trp Leu Ile Lys Ile
245 250 255

Leu Phe Tyr Tyr Met Ile Ser Leu Leu Phe Leu Phe Gly Asn Phe Tyr
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Thr Glu
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Ser Tyr Val Ser Ser Thr Val Gly Ser Trp Ser Val His Ser Ile Gln

35 40 45

Pro Leu Lys Arg Leu Thr Ser Lys Lys Arg Val Ser Glu Ser Ala Ala
50 55 60

Val Gln Cys Ile Ser Ala Glu Val Gln Arg Asn Ser Ser Thr Gln Gly
65 70 75 80

Thr Ala Glu Ala Leu Ala Glu Ser Val Val Lys Pro Thr Arg Arg Arg
85 90 95

Ser Ser Gln Trp Lys Lys Ser Thr His Pro Leu Ser Glu Val Ala Val
100 105 110

His Asn Lys Pro Ser Asp Cys Trp Ile Val Val Lys Asn Lys Val Tyr
115 120 125

Asp Val Ser Asn Phe Ala Asp Glu His Pro Gly Gly Ser Val Ile Ser
130 135 140

Thr Tyr Phe Gly Arg Asp Gly Thr Asp Val Phe Ser Ser Phe His Ala
145 150 155 160

Ala Ser Thr Trp Lys Ile Leu Gln Asp Phe Tyr Ile Gly Asp Val Glu
165 170 175

Arg Val Glu Pro Thr Pro Glu Leu Leu Lys Asp Phe Arg Glu Met Arg
180 185 190

Ala Leu Phe Leu Arg Glu Gln Leu Phe Lys Ser Ser Lys Leu Tyr Tyr
195 200 205

Val Met Lys Leu Leu Thr Asn Val Ala Ile Phe Ala Ala Ser Ile Ala
210 215 220

Ile Ile Cys Trp Ser Lys Thr Ile Ser Ala Val Leu Ala Ser Ala Cys
225 230 235 240

Met Met Ala Leu Cys Phe Gln Gln Cys Gly Trp Leu Ser His Asp Phe
245 250 255

Leu His Asn Gln Val Phe Glu Thr Arg Trp Leu Asn Glu Val Val Gly
260 265 270

Tyr Val Ile Gly Asn Ala Val Leu Gly Phe Ser Thr Gly Trp Trp Lys
275 280 285

Glu Lys His Asn Leu His His Ala Ala Pro Asn Glu Cys Asp Gln Thr
290 295 300

Tyr Gln Pro Ile Asp Glu Asp Ile Asp Thr Leu Pro Leu Ile Ala Trp
305 310 315 320

Ser Lys Asp Ile Leu Ala Thr Val Glu Asn Lys Thr Phe Leu Arg Ile
325 330 335

Leu Gln Tyr Gln His Leu Phe Phe Met Gly Leu Leu Phe Phe Ala Arg
340 345 350

Gly Ser Trp Leu Phe Trp Ser Trp Arg Tyr Thr Ser Thr Ala Val Leu
355 360 365

Ser Pro Val Asp Arg Leu Leu Glu Lys Gly Thr Val Leu Phe His Tyr
370 375 380

Phe Trp Phe Val Gly Thr Ala Cys Tyr Leu Leu Pro Gly Trp Lys Pro
385 390 395 400

Leu Val Trp Met Ala Val Thr Glu Leu Met Ser Gly Met Leu Leu Gly
405 410 415

Phe Val Phe Val Leu Ser His Asn Gly Met Glu Val Tyr Asn Ser Ser
420 425 430

Lys Glu Phe Val Ser Ala Gln Ile Val Ser Thr Arg Asp Ile Lys Gly
435 440 445

Asn Ile Phe Asn Asp Trp Phe Thr Gly Gly Leu Asn Arg Gln Ile Glu
450 455 460

His His Leu Phe Pro Thr Met Pro Arg His Asn Leu Asn Lys Ile Ala
465 470 475 480

Pro Arg Val Glu Val Phe Cys Lys Lys His Gly Leu Val Tyr Glu Asp
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Leu Ser Ser Leu Lys Gly Glu Glu Val Cys Ile Asp Gly Ile Ile Tyr
35 40 45

Asp Leu Gln Ser Phe Asp His Pro Gly Gly Glu Thr Ile Lys Met Phe
50 55 60

Gly Gly Asn Asp Val Thr Val Gln Tyr Lys Met Ile His Pro Tyr His
65 70 75 80

Thr Glu Lys His Leu Glu Lys Met Lys Arg Val Gly Lys Val Thr Asp
85 90 95

Phe Val Cys Glu Tyr Lys Phe Asp Thr Glu Phe Glu Arg Glu Ile Lys
100 105 110

Arg Glu Val Phe Lys Ile Val Arg Arg Gly Lys Asp Phe Gly Thr Leu
115 120 125

Gly Trp Phe Phe Arg Ala Phe Cys Tyr Ile Ala Ile Phe Phe Tyr Leu
130 135 140

Gln Tyr His Trp Val Thr Thr Gly Thr Ser Trp Leu Leu Ala Val Ala
145 150 155 160

Tyr Gly Ile Ser Gln Ala Met Ile Gly Met Asn Val Gln His Asp Ala
165 170 175

Asn His Gly Ala Thr Ser Lys Arg Pro Trp Val Asn Asp Met Leu Gly
180 185 190

Leu Gly Ala Asp Phe Ile Gly Gly Ser Lys Trp Leu Trp Gln Glu Gln
195 200 205

His Trp Thr His His Ala Tyr Thr Asn His Ala Glu Met Asp Pro Asp
210 215 220

Ser Phe Gly Ala Glu Pro Met Leu Leu Phe Asn Asp Tyr Pro Leu Asp
225 230 235 240

His Pro Ala Arg Thr Trp Leu His Arg Phe Gln Ala Phe Phe Tyr Met
245 250 255

Pro Val Leu Ala Gly Tyr Trp Leu Ser Ala Val Phe Asn Pro Gln Ile
260 265 270

Leu Asp Leu Gln Gln Arg Gly Ala Leu Ser Val Gly Ile Arg Leu Asp
275 280 285

Asn Ala Phe Ile His Ser Arg Arg Lys Tyr Ala Val Phe Trp Arg Ala
290 295 300

Val Tyr Ile Ala Val Asn Val Ile Ala Pro Phe Tyr Thr Asn Ser Gly
305 310 315 320

Leu Glu Trp Ser Trp Arg Val Phe Gly Asn Ile Met Leu Met Gly Val

325 330 335

Ala Glu Ser Leu Ala Leu Ala Val Leu Phe Ser Leu Ser His Asn Phe
340 345 350

Glu Ser Ala Asp Arg Asp Pro Thr Ala Pro Leu Lys Lys Thr Gly Glu
355 360 365

Pro Val Asp Trp Phe Lys Thr Gln Val Glu Thr Ser Cys Thr Tyr Gly
370 375 380

Gly Phe Leu Ser Gly Cys Phe Thr Gly Gly Leu Asn Phe Gln Val Glu
385 390 395 400

His His Leu Phe Pro Arg Met Ser Ser Ala Trp Tyr Pro Tyr Ile Ala
405 410 415

Pro Lys Val Arg Glu Ile Cys Ala Lys His Gly Val His Tyr Ala Tyr
420 425 430

Tyr Pro Trp Ile His Gln Asn Phe Leu Ser Thr Val Arg Tyr Met His
435 440 445

Ala Ala Gly Thr Gly Ala Asn Trp Arg Gln Met Ala Arg Glu Asn Pro
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Leu Thr Gly Arg Ala
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gacgcctatg atcgcatgat atttgctttc aattctgttg tgcacgttgt aaaaaacctg 120

agcatgtgta gctcagatcc ttaccgccgg tttcggttca ttctaatgaa tatatcaccc 180

gttactatcg tatttttatg aataatattc tccgttcaat ttactgattg tccgtcgacg 240

aattcgagct cggcgcgcca agctt 265

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tagctcagat ccttaccgcc ggtttcggtt cattctaatg aatatatcac ccgttactat 180

cgtatttta tgaataatat tctccgttca atttactgat tgtccgtcga cgaattcgag 240

ctcggcgcgc caagctt 257

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tagctcagat ccttaccgcc ggtttcggtt cattctaatg aatatatcac ccgttactat 180

cgtatttttа tgaataatat tctccgttca atttactgat tgtccgtcga cgaattcgag 240

ctcggcgcgc caagctt 257